An Efficient Production Process for Extracting Salivary Glands from Mosquitoes

Critical Review

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Project Summary

The goal of this project is to develop computer vision algorithms for mosquito detection and keypoint identification, and integrate said algorithms with a robotic system to assist automated mosquito dissection. Specifically, algorithms are being developed to identify the proboscis, head, and neck of mosquitos, and will be integrated with ROS to assist the robotic system. This project is being conducted in collaboration with Sanaria Inc. (Rockville, MD), a company which has developed a clinically effective live malaria vaccine in mosquitos. This project thus aims to assist the automated extraction of live malaria vaccine from mosquitos in order to facilitate large scale production of malaria vaccine.

Paper Summary

Relevance to Project

In this paper, Schrum et. al. develop a semi-automated method (SAMMS) to reduce operator training time and increase mosquitos dissected per hour, thereby increasing the production rate of the live malaria vaccine. The semi-autonomous methods used are primarily mechanical in nature, and the current robotic dissection system uses many of these methods in its workflow. The ultimate goal of this paper and the ultimate goal of our project are aligned in that both aim to increase the production rate of live malaria vaccine. Our project also builds on this paper's work to implement computer vision algorithms to further automate the mosquito dissection workflow established in this paper.

Key Results

The authors developed a semi-automated mechanical system which achieved two key benefits.

1. The system deskilled mosquito dissection. This reduced the training time required for technicians to achieve expertise in mosquito salivary gland extraction.
2. The system allowed for mosquitos to be dissected in batches of 20 instead of individually as previously done. This reduced dissection time per mosquito.

Both these results contributed to an increased throughput of mosquitos dissected per hour, which ultimately increases the production capacity of live malaria vaccine.

Introduction

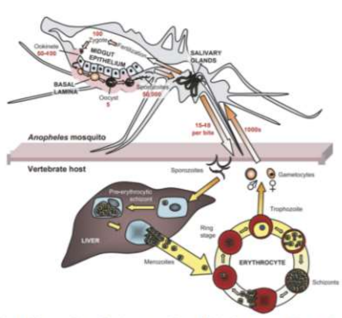

Fig 1. Life cycle of *plasmodium falciparum*. Note the location of the salivary glands in the mosquito. [1]

Malaria is widespread infectious disease, causing 216 million cases and 438,000 deaths in 2016. The parasite *Plasmodium falsiparum* (Pf) is the primary cause of death from malaria, infecting humans through mosquito vectors. Significant progress has been made developing a vaccine against Pf sporozoite (PfSPZ), but mass production is limited due to the difficulty of extracting the vaccine from the salivary glands of mosquitos, which is a time consuming and labour-intensive process. Prior efforts to partially or fully automate the extraction of salivary glands have been attempted, but current methods at the time of the paper's publication had a throughput of around 5-6 mosquitos per minute. Achieving this rate takes a technician several months of extensive training on average. Thus, with the goal of reducing the difficulty of mosquito dissection and increasing throughput of mosquitos to boost production, the authors of the paper designed their mechanical system.

Materials & Methods

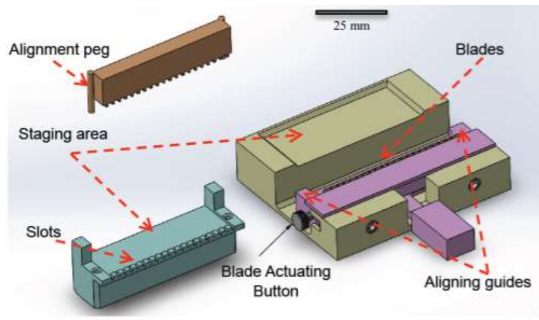

Fig. 3. Design of mosquito gland extraction apparatus, including the sorting cartridge (blue), the blade assembly (pink), squeezer (brown) and staging area (tan).

The authors developed a mechanical system called the "semi-automated mosquito microdissection system" (SAMMS) to simplify the mosquito dissection process. SAMMS is comprised of a sorting cartridge, automated blade assembly, a squeezer, and a staging area. The workflow for SAMMS is as follows. First, technicians place mosquitos on the staging area and align mosquito heads in the sorting cartridge. This is done by grasping the proboscis with tweezers and dragging the mosquito into a cartridge slot. Once mosquito necks are aligned, the blades are actuated to decapitate the mosquitos en masse. Finally, technicians extract mosquito salivary glands by using a squeezer comb to squeeze the glands out of mosquito thoraces onto a flat surface, where they can be collected with a pipette.

Fig. 4. Decapitation. (Top) Mosquitoes aligned in cartridge with heads between decapitation blades. (Bottom) Mosquitoes after decapitation.

The novel mechanical system was evaluated using two studies. The first study evaluated the mechanical system's throughput, measured by the number mosquitos a technician could dissect per minute. This was compared to the baseline without the mechanical system. The second study evaluated the training time required for new personnel to become proficient in mosquito gland extraction. The number of weeks required for proficiency was measured and compared to the previous baseline.

Results

SAMMS was able to markedly improve the mosquitos dissected per hour for operators. Figure 5 compares the previous throughput achieved by older techniques (2015 old) and the throughput achieved with SAMMS (2015 new, 2016 new). Training time for new operators to reach proficiency also decreased 10-15 fold when using SAMMS, from an average of 29 weeks to achieve 100 mosquitos per hour, to 1-3 days to achieve 600 mosquitos per hour. This also reduced variability in training times, which previously depended heavily on operator dexterity and hand-eye coordination. With dependable improvements in mosquito throughput, it became achievable for technicians to dissect 600 mosquitos per hour, which was an increase in throughput of at least two-fold.

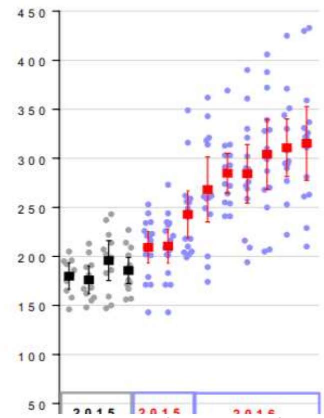

Figure 5. **Rate of dissection of mosquito salivary glands by manual dissection (abbreviated caption).** The new method of mosquito dissection (2015 new, 2016 new) saw an increase in mosquitos dissected per hour compared to the old methods (2015 old).

Table 2: Operation times and production rates for 8 operators using a proposed apparatus. (A) Minutes to align 20 mosquitoes; **(B)** Minutes for gland extrusion and collection. Total time for 20 mosquitoes is in minutes, and rate is mosquito throughput per hour.

	1	2	3	4	5	6	7	8	Avg
(A)	2.4	2.5	2.3	2.5	1.5	1.3	1.1	1.2	**1.9**
(B)	0.7	0.8	0.5	1.1	1.2	0.7	1.1	0.7	**0.8**
Total	3.1	3.3	2.8	3.6	2.7	2	2.2	1.9	**2.7**
Rate	393	364	429	338	444	600	545	649	**470**

Discussion

The need to increase the production capacity of live malaria vaccine drove the authors to innovate and develop SAMMS. The current mosquito decapitation system uses a batch size of 20, but the authors believe the optimal number to be around 40. A larger number may result in issues caused by mosquitos drying out and increased difficulty for the operator. Regardless, the design of SAMMS can be scaled to process an arbitrary number of mosquitos. The authors hope that the reduction in training time and increase in throughput will see the processing capacity of an operator using the system to double or triple compared to an operator performing single mosquito dissection in the future. The authors also note that the yield of PfSPZ from SAMMS harvested mosquitos is comparable to manual dissection, providing further validation for SAMMS. The authors briefly state that their previous experimentation to develop the techniques in SAMMS lead them to believe a vision-driven robotic system is the best approach for full automation.

Critique

In this paper, the authors clearly outline the need to increase production of PfSPZ vaccine, and present a method to increase throughput of mosquitos, thereby addressing the stated problem. The results indicate that SAMMS, the semi-automated mechanical system the authors propose, effectively reduces training time for new operators and increases mosquito throughput per operator. Furthermore, SAMMS is scalable to a large number of mosquitos, indicating its potential in future automated systems for mosquito dissection.

SAMMS is an excellent innovation that the authors presented in this paper. The simplicity of the design clearly deskilled the dissection process enough to reduce operator training time. Furthermore, it is designed in a way that can easily be scaled to any number of mosquitos, which is important for improving throughput in future workflows with increased automation. The success metrics the paper uses are also simple and clearly related to the end goal of the paper: increasing vaccine production. It is very easy for even laypeople with little background on malaria vaccine production to understand why SAMMS reducing training time and increasing throughput implies a higher vaccine production rate. Overall, the simplicity of SAMMS design and the study design is one of the greatest strengths of the paper.

Though simplicity is a great approach when simplifying information for the end reader, a limitation of this paper is that it may have focused too much on the two metrics of throughput and training time, and it could have provided more detail in other relevant areas. It would have been beneficial to have hard numbers comparing PfSPZ yield between manual and semi-automated harvesting rather than a couple sentences simply stating their equivalence in the discussion. Furthermore, it would have been useful to have more detail on shortcomings of this system. For example, the authors briefly mention that loading mosquitos into decapitation cartridges was a rate-limiting factor for new operators but did not provide any details why this may have been the case. Finally, the authors allude to the use of a vision-guided robotic system for a fully automated system in their discussion but provide scant details as to why they think this would be the best approach.

Ultimately, the authors of this paper did an excellent job clearly explaining a novel system that achieved significant increases in mosquito throughput and reductions in operator training time. This paper laid the foundation for the mechanical aspects of a fully automated robotic solution, which will hopefully provide further improvements in PfSPZ production. Our project aims to provide vision guidance, such as proboscis detection and neck localization, to further automate the process.

References

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