Synthetic Moth Antennae Fabricated as Preconcentrator for Odor Collection

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***Abstract*—Moths do not employ any of the techniques modern preconcentrators use to extract odors from the air yet they are able to sense pheromone particles in concentrations of parts per billion. Their odor grabbing abilities originate from the complex structure of their antennae; however, this unique anatomical structure has never been replicated to scale. We analyze natural moth olfaction systems to find commonalities in the antennae across species. We create 1:1 scale antennae mimics that contains these commonalities and overcome the unique fabrication challenges of the small scale and hierarchical nature of the antennae using nanoscale 3D printing. The comparative biology and fabrication techniques from this study may inspire ideas for fabricating improved filters and chemical sensors.**

I. Introduction

Chemical communication is a means by which insects and other animals find food sources and mates. The use of pheromones is common across the animal kingdom but moths have long been known as masters of chemical communication.[1][2] Some such moths have been observed to locate females across distances from 500 m to over 4 km away, tracking concentrations of pheromone in parts per billion[3][4][5] which is better than any current artificial sensor of their size.[6][7]

The presence of antennae used to sniff the area is a common feature of invertebrates, appearing in lobsters, crabs, and flying insects.[8][9] Male moths have perhaps the most spectacular antenna with their surface area greatly increased by its unique structure.[10] Previous workers have shown the structure of 152 moth species, but little work has been done on comparing specific features of moths with plumose antennae towards finding universal principles of antenna design.[11]

The goal of the structure of the moth antennae is to serve as a natural, passive preconcentrator to trap the analytes. Moths do not have the ability to preconcentrate the air using conventional synthetic means such as heating pulses. A major issue with current preconcentrators is the time it takes to collect enough odor to get a good measurement. [12] [13] Typical sensors take on the order of minutes to identify a gas, however moths can sense a pheromone in less than a second. [14] In applications such as in the detection of explosives, the time frame needs to be decreased by an order of magnitude from what is currently possible. [15] By studying natural olfaction systems, we should be able to decrease the amount of time required by the preconcentrator to collect the analyte.

The goal of this study is to analyze natural olfaction structure of the male moth antenna and identify any trends which make it so effective at odor collection. We approach the problem of reducing the time to concentrate an odor by studying the natural particle collectors with comparative biology and then replicating the unique structure with nanofabrication. This newly fabricated structure can then be used for fluid dynamic analysis to observe physically how odors land on the antennae.

II. Results

In the 31 unique species of moths caught, we observe a hierarchy of three levels of branching, which we call the stalk (tier 1), branch (tier 2), and sensilla (tier 3) as shown in Figure 1. The characteristic diameters at each these levels are 130 microns, 40 microns, and 2 microns in diameter respectively. Surprisingly, each level also has comparably similar ratios of length to diameter, from 15-80. Across all the antennae measured, the branches consistently extend in angle of 50 $\pm$ 15 degrees. This consistency in angle is surprising because previous literature on particle collection predicts the branches to be angled at 90 degrees in order to provide the most surface area as possible to extract the pheromones from the air. [16] The consistency of branch angles suggests a new avenue to study involving particle collection.

When deceased, natural moth antennae quickly become too brittle to perform deposition tests. Therefore, to test the collection ability of the antennae structure, synthetic mimics must be created with the same dimensions and structure as the natural antennae. The small scale, hierarchical nature, and high aspect ratio features of the antennae provide a unique fabrication challenge that cannot be solved using traditional methods. However, through the procedure provided in the materials and methods section, we are able to duplicate not only the shape, but also approximate the surface features of a natural antennae's sensilla and branch as shown in Figure 2. The surface of the natural antennae has ridges of approximately 0.1 microns as seen in Figure 1e and the method used to create the 3D printed antennae generates a surface roughness of about 100 nm-peak-to-valley[17], Figure 2f.

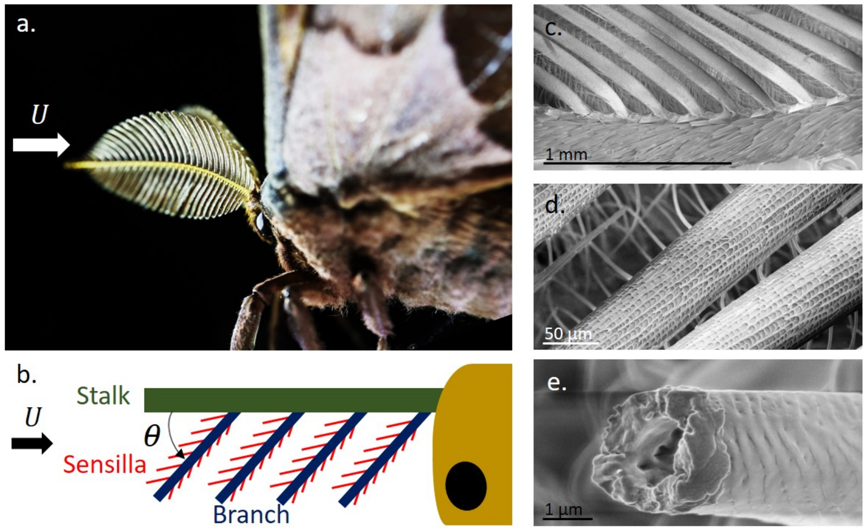

Fig. 1. a. *Antheraea Polyphemus* with complex antennae b. Schematic of antennae hierarchy levels c. Antennae branches extruding from stalk d. Antennae sensilla extruding from branch e. Sensilla cut to reveal cylindrical cross section and 0.1 micron ridges

By gold sputtering and connecting silver electrodes to the ends of the fabricated antennae, modified Electroantennography can be performed to measure the particle deposition response similar to the process of Kuwana et. al. [6] In their process, the silkmoth's antennae became unusable after 80 minutes of experimentation, but this newly fabricated antennae should have no time constraint. This lack of time constraint is important for the future fluid dynamic analysis of the odor collection of the antennae.

III. Experimental Methods

A. Moth Collection

We obtained approximately one hundred North American moth specimens, spanning 31 species. Approximately two hundred moths were caught in the Nantahala mountain region in North Carolina of which 10 unique species of male moths could be identified. The remaining 21 species were obtained from the department of Biology at the University of Connecticut and all moths were examined using a 3D scanning confocal microscope. The captured moths are anesthetized using a five second burst of compressed carbon dioxide then euthanized via freezing and sorted based on antennae type.

B. Antennae Branch Angle Measurements

After carefully cutting one antenna, from its base, off of a moth sample, it is mounted onto aluminum wire to allow for easier manipulation while imaging. The wire is dipped into Loctite UV curing glue first, then carefully stuck to the main branch of the antenna. The accompanying handheld UV light is then used to cure the glue and secure the antenna to the wire. The other end of the wire is inserted into a ball of modeling compound to keep the antenna from touching the microscope stage, but also to allow for easy rotation of the antenna.

All imaging uses an Olympus LEXT 3D Material Confocal Microscope with fixed laser wavelength of 405 nm and resolution of 120nm resulting in a collapsed 3 dimensional image with better resolution than light microscopy alone. An initial, low resolution, z-stack is done with a low powered laser using a small focal plane, to ensure that each branch of the antennae lies in the same z-plane so the angle measurements are accurate. Reflection of the laser is collected to determine the surface topography of the object being imaged. Light microscopy is used at the previously determined topological points creating a z-stack of pixel values which are ultimately displayed. In this way, a 2 dimensional image of great quality is created from a small 3 dimensional object.

C. Antennae Mimic Nanofabrication

The 1:1 scale antennae mimic is created using the Nanoscribe Photonic Professional laser lithography system with a two-photon polymerization process and SU-8 photoresist. The entire structure is fixed to the tip of a 30 gauge hypodermic needle with a 90-degree syringe pipetting blunt end in order to allow for easy portability and handling of the airborne molecule collection device.

The orienting laser on the NanoScribe is initially focused on the top of the needle's contour and then manually lowered by a distance equal to the radius of the needle. This new Z location is set as the starting height for the print job and the piezo actuated stage is programmed to move as if printing on a flat surface at this height. The support for the antennae uses 100x100x100 micrometer cubes with the center of each cube lying along an arc with a radius equal to that of the needle. This alignment allows for the laser to harden the resin that penetrates into the surface features of the needle and provide a solid foundation for the rest of the antennae. 5 individual antennae mimics have been successfully created with branching angles from 30 to 75 degrees in increments of 15 degrees to be used in future particle collection tests.

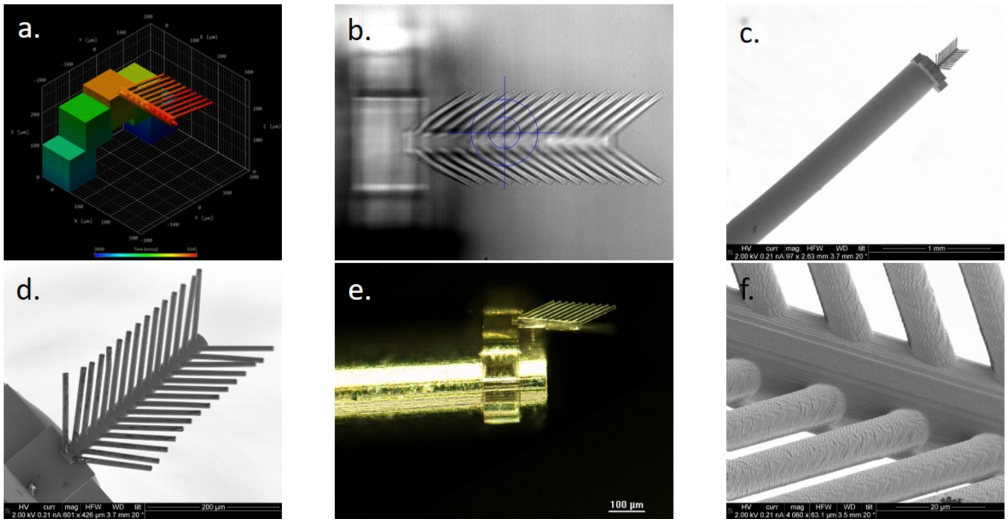

Fig. 2. a. Antennae structure subdivided into 200 nm cube sections to grab onto needle and avoid overlapping features b. Antennae fabrication in progress c-e. Completed sensilla and branch on needle tip f. Surface structure of print comparable to natural chitin

The NanoScribe 3D laser lithography system prints in 100^3 μm^3 increments, therefore the design is broken into similar sized segments which are mated together in the NanoScribe software. For example, the first five branches on the fabricated antennae are modeled as a separate part file from the next 5 as indicated in the different colors of Figure 1a.

One common challenge in fabrication of high aspect ratio structures is the agglomeration of adjacent pillars when spaced too closely together. The spacing between the centerline of each natural moth antennae branch is approximately equivalent to twice the diameter of each branch. When reconstructing the antennae mimic, branch spacing of two diameters is enough room for the uncured SU-8 to be removed between branches in all antennae with over 30 degree branching angles.

IV. Conclusion

Through this study, we analyzed the natural olfaction structures contained on male moths which are able to quickly identify pheromone chemicals from the air. We found the structures to have three levels of hierarchy with the second level extending at a consistent 45 $\pm$ 15 degree angle. We successfully replicated the structure at a 1:1 scale using a laser lithography system with two-photon polymerization. Coupled together, the comparative biology and fabrication method introduce a new approach to create a preconcentrator which relies on the passive geometry rather than active control.

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References

[1] T. D. Wyatt, "Fifty years of pheromones," *Nature*, vol. 457, no. 7227, pp. 262–263, 2009.

[2] Wyatt, *Pheromones and animal behaviour: communication by smell and taste*. Cambridge University Press, 2003.

[3] Y. Kuwana and R. Kanzaki, "Synthesis of the pheromone-oriented behaviour of silkworm moths by a mobile robot with moth antennae as pheromone sensors," *Biosensors and Bioelectronics*, vol. 14, no. 2, pp. 195–202, 1999.

[4] P. Rau and N. M. Rau, *The Sex Attraction and Rhythmic Periodicity in Giant Saturnid Moths*, 1929.

[5] C. Wall and J. Perry, "Range of action of moth sexattractant sources," *Entomologia experimentalis et applicata*, vol. 44, no. 1, pp. 5–14, 1987.

[6] Kuwana and H. Miura, "Synthesis of pheromone-oriented emergent behavior of a silkworm moth," in *Intelligent Robots and Systems' 96, IROS 96*, vol. 3. IEEE, Conference Proceedings, pp. 1722–1729.

[7] J. A. Riffell, L. Abrell, and J. G. Hildebrand, "Physical processes and real-time chemical measurement of the insect olfactory environment," *Journal of chemical ecology*, vol. 34, no. 7, pp. 837–853, 2008.

[8] M. Koehl and P. A. Moore, "Lobster sniffing: antennule design and hydrodynamic filtering of information in an odor plume," *Science*, vol. 294, no. 5548, pp. 1948–1951, 2001.

[9] L. D. Waldrop, L. A. Miller, and S. Khatri, "A tale of two antennules: the performance of crab odour-capture organs in air and water," *Journal of The Royal Society Interface*, vol. 13, no. 125, p. 20160615, 2016.

[10] D. Schneider, "Insect antennae," *Annual review of entomology*, vol. 9, no. 1, pp. 103–122, 1964.

[11] M. R. Symonds, T. L. Johnson, and M. A. Elgar, "Pheromone production, male abundance, body size, and the evolution of elaborate antennae in moths," *Ecology and evolution*, vol. 2, no. 1, pp. 227–246, 2012.

[12] R. Gutierrez and A. Hierlemann, "Adaptive microsensor systems," *Annual Review of Analytical Chemistry*, vol. 3, pp. 255–276, 2010.

[13] G. Korotcenkov and B. Cho, "Engineering approaches for the improvement of conductometric gas sensor parameters: Part 1. improvement of sensor sensitivity and selectivity (short survey)," *Sensors and Actuators B: Chemical*, vol. 188, pp. 709–728, 2013.

[14] M. Stengl, "Pheromone transduction in moths," *Design principles of sensory receptors*, p. 77, 2013.

[15] J. I. Steinfeld and J. Wormhoudt, "Explosives detection: a challenge for physical chemistry," *Annual Review of Physical Chemistry*, vol. 49, no. 1, pp. 203–232, 1998.

[16] L. A. Spielman, "Particle capture from low-speed laminar flows," *Annual review of fluid mechanics*, vol. 9, no. 1, pp. 297–319, 1977.

[17] X. Zhou, Y. Hou, and J. Lin, "A review on the processing accuracy of two-photon polymerization," *AIP Advances*, vol. 5, no. 3, p. 030701, 2015.