Journal of Visualized Experiments

Flying Insect Detection and Classification with Inexpensive Sensors

--Manuscript Draft--

Manuscript Number:	JoVE52111R3
Full Title:	Flying Insect Detection and Classification with Inexpensive Sensors
Article Type:	Methods Article - JoVE Produced Video
Keywords:	Flying Insect Detection; Automate Insect Classification; Pseudo-Acoustic Optical Sensors; Bayesian Classification Framework; Flight Sound; Flight Activity Circadian Rhythm
Manuscript Classifications:	12.1.100: Classification; 12.1.280: Data Collection
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Abstract:	An inexpensive, noninvasive system that could accurately classify flying insects would have important implications for entomological research, and allow for the development of many useful applications in vector control for both medical and agricultural entomology. Given this, the last sixty years have seen many research efforts devoted to this task. To date, however, none of this research has had a lasting impact. In this work, we show that pseudo-acoustic optical sensors can produce superior data; that additional features, both intrinsic and extrinsic to the insect's flight behavior, can be exploited to improve insect classification, that a Bayesian classification approach allows to efficiently learn classification models that are very robust to over-fitting, and a general classification framework allows to easily incorporate arbitrary number of features. We demonstrate the findings with large scale experiments that dwarf all previous works combined, as measured by the number of insects and the number of species considered.
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Dear Sir/Madam,

The history of humankind is intimately connected to insects. Given the importance of insects in human affairs, it is surprising that computer science has not had a larger impact in entomology. We believe that recent advances in sensor technology and machine learning techniques are about to change this and a field of computational entomology will emerge. In particular, we proposed a system that can recognize the sex and species of insects as they fly past a light beam. In the system, we used a custom-designed inexpensive sensor to automatically collect insect flying sounds, and we designed a software to automatically classify the insects based on their flying sounds. We have very impressive results on the classification and we would like to show the world how we do this.

So far, we have made all code, data and supplemental materials freely available at our webpage (*https://sites.google.com/site/insectclassification/*), and we give sensors for free to researchers interested in it. We believe our work will provide researchers worldwide robust tools to accelerate their research.

A video is the best way to present this paper, because:

1. The readers can *hear* the insect sounds in the video.
2. The readers will better understand this system by watching a video. As we continue giving a complete sensor system to research entomologists who request one, we need to explain how to use it. The best way to show people how to use the system as a tool is a video presentation.
3. The JOVE paper http://www.jove.com/video/2157/assaying-locomotor-activity-to-study-circadian-rhythms-sleep is a good model for how we would present this work.

We would like to thank Editor *Elizabeth Sheeley* for inviting and assisting us with this paper.

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Best regards,
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KEYWORDS
Flying Insect Detection, Automatic Insect Classification, Pseudo-Acoustic Optical Sensors, Bayesian Classification Framework, Flight Sound, Flight Activity Circadian Rhythm

SHORT ABSTRACT
We proposed a system that uses inexpensive, noninvasive pseudo-acoustic optical sensors to automatically and accurately detect, count and classify flying insects based on their flying sound.

LONG ABSTRACT
An inexpensive, noninvasive system that could accurately classify flying insects would have important implications for entomological research, and allow for the development of many useful applications in vector control for both medical and agricultural entomology. Given this, the last sixty years have seen many research efforts devoted to this task. To date, however, none of this research has had a lasting impact. In this work, we show that *pseudo-acoustic optical sensors* can produce superior data; that additional features, both intrinsic and extrinsic to the insect's flight behavior, can be exploited to improve insect classification, that a Bayesian classification approach allows to efficiently learn classification models that are very robust to over-fitting, and a general classification framework allows to easily incorporate arbitrary number of features. We demonstrate the findings with large scale experiments that dwarf all previous works combined, as measured by the number of insects and the number of species considered.

INTRODUCTION
The idea of automatically classifying insects using the incidental sound of their flight dates back to the earliest days of computers and commercially available audio recording equipment[1]. However, little progress has been made on this problem in the intervening decades. The lack of progress in this pursuit can be attributed to several related factors.

First, the lack of effective sensors has made data collection difficult. Most efforts to collect data have used acoustic microphones[2-5]. Such devices are extremely sensitive to wind noise and to ambient noise in the environment, resulting in very sparse and low-quality data.

Second, compounding the data quality issues is the fact that many researchers have attempted to learn very complicated classification models, especially neural networks[6-8]. Attempting to learn complicated classification model with a mere tens of examples is a recipe for over-fitting.

Third, the difficultly of obtaining data has meant that many researchers have attempted to build classification models with very limited data, as few as 300 instances[9] or less. However, it is known that for building classification models, more data is better[10-12].

This work addresses all three issues. Optical (rather than *acoustic*) sensors can be used to record the "sound" of insect flight from meters away, with complete invariance to wind noise and ambient sounds. These sensors have allowed the recording of millions of labeled training instances, far more data than all previous efforts combined, and thus help avoid the over-fitting that has plagued previous research efforts. A principled method is shown that allows the incorporation of additional information into the classification model. This additional information can be as quotidian and as easy-to-obtain as the time-of-day, yet still produce significant gains in accuracy. Finally, it is demonstrated that the enormous amounts of data we collected allow us to take advantage of "*The unreasonable effectiveness of data*"[10] to produce simple, accurate and robust classifiers.

In summary, flying insect classification has moved beyond the dubious claims created in the research lab and is now ready for real-world deployment. The sensors and software presented in this work will provide researchers worldwide robust tools to accelerate their research.

PROTOCOL

1. Insect Colony and Rearing

1.1. Mosquito Colony and Rearing

1.1.1. Rear adult mosquitoes from lab colonies, which originated from wild caught individuals.

1.1.2. Rear mosquito larvae in enamel pans under standard laboratory conditions (27°C, 16:8 hr light:dark [LD] cycle with 1 hr dusk/dawn periods), and feed them *ad libitum* on a mixture of ground rodent chow and Brewer's yeast (3:1, v:v).

1.1.3. Collect mosquito pupae into 200-mL cups, and place them into experimental chambers. Alternatively, aspirate the adult mosquitoes into experimental chambers within 1 week of emergence. Make sure each experimental chamber contains 20 to 40 individuals of the same species/sex.

1.1.4. Feed adult mosquitoes *ad libitum* on a 10% sucrose and water mixture. Replace food weekly.

1.1.5. Moisten cotton towels twice a week and place them on top of the experimental chambers to maintain humidity within the cage,. In addition, place a 200-ml cup of tap water in the chamber at all times.

1.1.6. Maintain the experimental chambers on a 16:8 hr light:dark [LD] cycle, 20.5-22°C and 30-50% RH for the duration of the experiment.

1.2. Fly Colony and Rearing

1.2.1. Rear *Musca domestica* from a lab colony, derived from wild caught individuals. Catch wild *Drosophila simulans* individuals and rear them in the experimental chambers.

1.2.2. Rear *Musca domestica* larvae in plastic tubs under standard laboratory conditions (12:12 hr light:dark [LD] cycle, 26°C, 40% RH) in a mixture of water, bran meal, alfalfa, yeast, and powdered milk. Rear *Drosophila simulans* larvae in plastic cups and feed them *ad libitum* on a mixture of rotting fruit.

1.2.3. Aspirate adult *Musca domestica* into experimental chambers within 1 week of emergence. Rear adult *Drosophila simulans* directly in the experimental chambers. Make sure each experimental chamber contains 10-15 individual *Musca domestica* or 20-30 individual *Drosophila simulans.*

1.2.4. Feed adult *Musca domestica ad libitum* on a mixture of sugar and low-fat dried milk, with free access to water. Feed adult *Drosophila simulans ad libitum* on a mixture of rotting fruit. Replace food weekly.

1.2.5. Maintain experimental chambers on a 16:8 hr light:dark [LD] cycle, 20.5-22°C and 30-50% RH for the duration of the experiment.

2. Record Flying Sounds in Experimental Chambers

2.1. Experimental chamber setup.

Note: An experimental chamber is a cage designed in our lab to do the data collection. The sensor is inexpensive. When built in bulk, a set up could be manufactured for less than $10.

2.1.1. Construct an experimental chamber, either of the larger size of 67 cm L x 22 cm W x 24.75 cm H, or of the smaller size of 30 cm L x 20 cm W x 20 cm H. The experimental chamber, consists of a phototransistor array and a laser line pointing at the phototransistor array.

NOTE: Additionally, the chamber consists of kritter keepers that are modified to include the sensor apparatus as well as a sleeve attached to a piece of PVC piping to allow access to the insects.

2.1.2. Connect the phototransistor array to an electronic board. The output of the electronic board feeds into a digital sound recorder and is recorded as audio data in the MP3 format. See the logic design of the sensor in Figure 1. II and a physical version of the chamber in Figure 1.I.

2.1.3. Modify the lids of the experimental chambers with a piece of mesh cloth affixed to the inside in order to prevent escape of the insects, see **Error! Reference source not found.**.I.

NOTE: When an insect flies across the laser beam, its wings partially occlude the light, causing small light fluctuations. The light fluctuations are captured by the phototransistor array as changes in current, and the signal is filtered and amplified by the custom designed electronic board.

2.2. Set Up the System to Record Flying Sounds

2.2.1. Connect the experimental chamber to a power supply. Turn on the power.

2.2.2. On the experimental chamber, find the laser lights and photoarray. Align the laser lights to the photoarray. To get a good alignment, adjust the photoarray using the magnets attached to the photoarray until the laser fall centered on all the individual photodiodes.

2.2.3. Perform two sanity checks to make sure the system is properly set up.

NOTE: The first step is to make sure that the system is powered, all wires are properly connected and the laser is pointing at the photo array. The second step is to further check if the alignments of the laser and the photo array are good enough to capture the sound of the insect wingbeats.

2.2.3.1. Plug in headphones (rather than the recorder) into the audio jack. Plunge hand in and out near the laser source end. Make sure the laser light is on the hand as the hand moves. Listen to the headphone as the hand goes in and out. If the sound of the hand movements is heard, the sensor can capture the sound produced by the movement of big objects. In that case, move on to the next check; otherwise, check if the headphone is properly connected and whether the laser is pointing at the photoarray. Adjust accordingly until the sound of the hand movement can be heard.

2.2.3.2. Attach a string to an automatic toothbrush. Turn on the toothbrush, and plunge the string in and out near the laser source end. Make sure the laser light is on the string as it moves. If the sound of the string movements is heard, the system can capture the sound produced by the movement of tiny objects, and is ready for collect insect sounds; otherwise, go back to step **Error! Reference source not found.** to re-align the laser lights and the photoarray.

2.2.4. After the system is properly set up, add insects to the cage, and close the lid.

2.3. Data Collection: Record Insect Flight Sounds

2.3.1. Turn on the recorder and make a voice annotation that includes the following information: name of the species in the cage, age of the insects, date and time, current ambient room temperature and relative humidity. Pause the recording.

2.3.2. Connect the recorder to the system, and resume the recording. Leave the recorder to record for 3 days, then stop the recording.

2.3.3. Download the data from the recorder into a new folder on a PC. Empty the recorder by deleting the data.

2.3.4. Repeat the above recording process, until the remaining insects have died off and there are no more than 5 insects left alive in the cage.

3. Sensor Data Processing and Flying Sound Detection

3.1. Use Software to Detect Flying Sound

NOTE: The software (detection algorithm) is much faster than real-time. It takes less than three hours to process a recording session, i.e., three days data, on a standard machine with Intel(R) Core ™ CPU at 2.00GHz and 8GB RAM.

3.1.1. For each folder containing data from a recording session, run the detection software (Chen 2013) to detect insect sounds. To run the software, open Matlab, and type "circandian_wbf (*dataDir*)" in the command window, where *dataDir* is the directory of the recording data. Press "Enter" to start.

NOTE: Download circadian_wbf from reference # 15.

3.1.2. Wait until the algorithm terminates, then check the detection results. The algorithm outputs all the detected insect sounds in a new folder named "*dataDir*_extf", where *dataDir* is the same as in the previous step. Each sound file is a one-second long audio originally extracted from the raw recording, with a digital filter applied to remove noise. The occurrence time of each detected sound is saved in a file named "*dataDir*_time.mat". Observe the example of a detected insect sound in Figure 2.

3.2. Detection Algorithm

3.2.1. Use a 0.1 second long sliding window to slide through the recording. The sliding window starts from the beginning of the recording. For each window, follow the steps below.

3.2.1.1. Compute the fundamental frequency of the current window.

3.2.1.2. If the fundamental frequency is within the range of 100 Hz to 1200 Hz, then do the following:

3.2.1.2.1. Extract the one-second long audio clip centering at the current window from the recording; apply a digital filter to remove the noise in the clip and save the filtered audio into the folder "*dataDir*_extf".

3.2.1.2.2. Save the occurrence time of the current window into the file "*dataDir*_time".

3.2.1.2.3. Move the sliding window to the point that immediately after the extracted audio.

3.2.1.3. Otherwise (If the fundamental frequency is NOT within the range of 100 Hz to 1200 Hz), simply move the sliding window 0.01 second forward.

3.2.2. Repeat the process until the sliding window reaches the end of the recording.

4. Insect Classification

4.1. Bayesian Classification Using Just the Flying Sound

Note: Bayesian classifier is a probabilistic classifier that classify an object to its most probable class.

4.1.1. Sound Feature Computation

4.1.1.1. For each insect sound, compute the frequency spectrum of the sound using the Discrete Fourier Transform (DFT). Truncate the frequency spectrum to include only those corresponding to the frequency range of 100 Hz to 2,000 Hz. The truncated frequency spectrum is used in the classification as the representative of the insect sound.

NOTE: The DFT is an algorithm that transforms signals in time domain to the frequency domain. It is a built-in function in most programming libraries, and can be called in the program with just one line of code.

4.1.2. Train a Bayesian classifier

4.1.2.1. Use the kNN density estimation approach[14] to learn the posterior probability distribution using the sound feature. With the kNN approach, the training phase is to build a training dataset.

4.1.2.1.1. Randomly sample a number of insect sounds from the data collected for each species of insects.

4.1.2.1.2. Follow the steps in Section 4.1.1 and compute the truncated frequency spectrum for each sampled sound. The truncated spectrums together with the samples' class labels (insect species name) composed the training dataset.

4.1.3. Use the Bayesian classifier to classify an unknown insect

4.1.3.1. Compute the truncated frequency spectrum of the unknown insect sound.

4.1.3.2. Compute the Euclidean distance between the truncated spectrum of the unknown object and all the truncated spectrums in the training dataset.

4.1.3.3. Find the top *k* (*k* = 8 in this paper) nearest neighbors of the unknown object in the training dataset. Compute the *posterior* probability of the unknown insect sound belonging to a class C_i as the fraction of the top *k* nearest neighbors which are labeled as class C_i.

NOTE: Suppose there are n_i neighbors labeled as C_i, then the posterior probability of class C_i is $\frac{n_1}{k}$.

4.1.3.4. Classify the unknown object to the class that has the highest *posterior* probability.

4.2. Add a Feature to the Classifier: Insect Flight Activity Circadian Rhythm

4.2.1. Learn the class-conditioned distributions of the occurrence time of insect sound, that is, the flight activity circadian rhythm for each species of insects.

4.2.1.1. Obtain the occurrence time of each sound from the detection results (c.f. Section 3.2).

4.2.1.2. For each species, build a histogram of the insect sound occurrence time.

4.2.1.3. Normalize the histogram so that the area of the histogram is one. The normalized histogram is the flight activity circadian rhythm of a species. It tells the probability of observing an insect of that species at different time.

4.2.2. Classify an unknown insect sound by combining the insect sound and the flight activity circadian rhythm

4.2.2.1.1. Given the occurrence time of the unknown sound, obtain the probability of observing an insect of class C_i at the time based on the flight activity circadian rhythm of class C_i.

NOTE: The flight activity circadian rhythm is a probability distribution. It is an array specifying the probability to detect an insect sound at each time of the day. So once a time is given, one can simply check the array to get the probability.

4.2.2.1.2. Follow the steps in section 4.1.2 to compute the *posterior* probability that the unknown sound belongs to class C_i using the sound features. Multiply the *posterior* probability to the results from the previous step to get the new *posterior* probability.

4.2.2.1.3. Classify the unknown sound to the class that has the highest new *posterior* probability.

4.3. Add One More Feature to the Classifier: Insect Geographic Distribution

4.3.1. Learn the geographic distribution of insects, either from data collected in the past, relevant documents, or simply the experience from field technicians. For demonstration purpose, use a simulation of the graphic distribution, as shown in Figure 7.

4.3.2. Classify an unknown insect sound using flying sound and the two additional features

4.3.2.1. Given the location where the insect sound was intercepted, compute the probability of observing an insect from class C_i at the location using the graphic distribution of species C_i.

4.3.2.1.1. Follow steps in section 4.2.2 and compute the *posterior* probability that the unknown sound belongs to class C_i using the sound features and the flight activity circadian rhythms. Multiply it to the results from the previous step to get the new *posterior* probability.

4.3.2.2. Classify the unknown sound to the class that has the highest new *posterior* probability.

4.4. A General Framework for Adding Features

4.4.1. Consider the Bayesian classifier that uses just the sound features as the primary classifier. Follow the steps below to add new features to the classifier.

4.4.1.1. In the training phase, learn the class-conditioned density functions of the new feature.

4.4.1.2. In the classification phase, given the new feature of the unknown sound, compute the probability of observing the feature in a class C_i using the density functions learned in the previous step. Multiply this probability to the previous *posterior* probability of the unknown sound belonging to classs C_i , which were computed based on just the odd features, to obtain the new *posterior* probability. Classify the unknown object to the class that has highest new *posterior* probability.

REPRESENTATIVE RESULTS

Two experiments are presented here. For both experiments, the data used were randomly sampled from a dataset that contains over 100,000 objects.

The first experiment showed the ability of the proposed classifier to accurately classify different species/sexes of insects. As the classification accuracy depends on the insects to be classified, a single absolute value for classification accuracy will not give the reader a good intuition about the performance of the system. Instead, rather than reporting the classifier's accuracy on a fixed set of insects, the classifier was applied to datasets with an incrementally increasing number of species, and therefore increasing classification difficulty.

The dataset began with just two species of insects; then at each step, one more species (or a single *sex* of a sexually dimorphic species) was added and the classifier was used to classify the increased number of species (the new dataset). A total of ten classes of insects (different sexes from the same species counting as different classes) was considered, with 5,000 exemplars in each class.

The classifier used both *insect-sound* (frequency spectrum) and *time-of-intercept* for classification. Table 1 shows the classification accuracy measured at each step and the relevant class added at that step.

According to Table 1, the classifier achieves more than 96% accuracy when classifying no more than five species of insects, significantly higher than the default rate of 20% accuracy. Even when the number of classes considered increases to ten, the classification accuracy is never lower than 79%, again significantly higher than the default rate of 10%. Note that the ten classes are not easy to separate, even by human inspection. Among the ten species, eight of them are mosquitoes; six of them are from the same genus.

The second experiment is to show the performance of the system to sex flying insects, specifically, to distinguish male *Ae*. *aegypti* mosquitoes from the females. In the first part, assume that the misclassification cost of misclassifying males as females is the same as the cost of misclassifying females as males. With this assumption, the classification results are shown in Table 2.I. The classification accuracy to sex *Ae*. *aegypti* is about 99.4%.

In the second part, assume the cost is not asymmetric, that misclassification of female as male cost much more than the reverse. With this assumption, the decision threshold of the classifier was changed to reduce the number of high-cost misclassifications. With the threshold properly adjusted, the classification results in Table 2.II were achieved. Of 2,000 insects in the experiment, twenty-two males, and *zero* females were misclassified.

FIGURE AND TABLE LEGENDS:

Figure 1: I) One of the cages used to gather data. II) A logical version of the sensor setup with the components annotated

Figure 2: I) An example of a one-second audio clip containing a flying generated by the sensor. The sound was produced by a female *Cx*. *stigmatosoma*. The insect sound is highlighted in red/bold. II) The insect sound that is cleaned and saved into a one-second long audio clip by centering the insect signal and padding with 0s elsewhere. III) The frequency spectrum of the insect sound obtained using Discrete Fourier Transform.

Figure 3: A Bayesian network that uses a single feature for classification

Figure 4: A Bayesian network that uses two independent features for classification

Figure 5: The flight activity circadian rhythms of *Cx. stigmatosoma* (female), *Cx. tarsalis* (male), and *Ae. Aegypti* (female), learned based on observations generated by the sensor that were collected over one month

Figure 6: A Bayesian network that uses three independent features for classification

Figure 7: The assumptions of geographic distributions of each insect species and sensor locations in the simulation to demonstrate the effectiveness of using location-of-intercept

feature in classification

Figure 8: The general Bayesian network that uses n features for classification, where n is a positive integer

Table 1: Classification accuracy with increasing number of classes

Table 2: (I) The confusion matrix for sex discrimination of *Ae*. *aegypti* mosquitoes with the decision threshold for female being 0.5 (i.e., same cost assumption). (II) The confusion matrix of sexing the same mosquitoes with the decision threshold for female being 0.1.

DISCUSSION:

The sensor/classification framework described here allows the inexpensive and scalable classification of flying insects. The accuracies achievable by the system are good enough to allow the development of commercial products and to be a useful tool for entomological research.

The ability to use inexpensive, noninvasive sensors to accurately and automatically classify flying insects would have significant implications for entomological research. For example, by deploying the system in the field to count and classify insect vectors, the system can provide real-time counts of the target insects, producing real-time information that can be used to plan intervention/suppression programs to combat malaria. Moreover, the system can automatically separate insects by sex, and thus it can be used to free entomologists working on the Sterile Insect Technique[15] from the tedious and time-consuming task of manually sexing the insects.

In using this system, the most critical step is to properly set up the sensor for data collection. If the laser and the photo array are not properly aligned, the data will be very noisy. After the insects are placed in the cage, the photo array is fine-tuned using the magnetics outside the cage. Note that flashing lights, camera flashes and vibrations near the cages will introduce noise to the data. Therefore, to obtain clean data, place the cage in a dark room, and wherever necessary, place dry towels under the cages to avoid vibration.

The classifier presented in this work used just two additional features. However, there may be dozens of additional features that could help improve the classification performance. As the potential features are domain and application specific, the users could choose features based on the applications. The general framework of the classifier allows users to easily add features to the classifier to improve the classification performance.

To encourage the adoption and extension of our ideas, we are making all code, data, and sensor schematics freely available at the UCR Computational Entomology Page[16]. Moreover, within the limits of our budget, we will continue our practice of giving a complete system (as show in Figure 1) to any research entomologist who requests one.

ACKNOWLEDGMENTS

We would like to thank the Vodafone Americas Foundation, the Bill and Melinda Gates Foundation and São Paulo Research Foundation (FAPESP) for funding this research, and the many faculties from the Department of Entomology at UCR that offered advice and expertise.

DISCLOSURES

The authors declare that they have no competing financial interests.

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Figure1-sensor

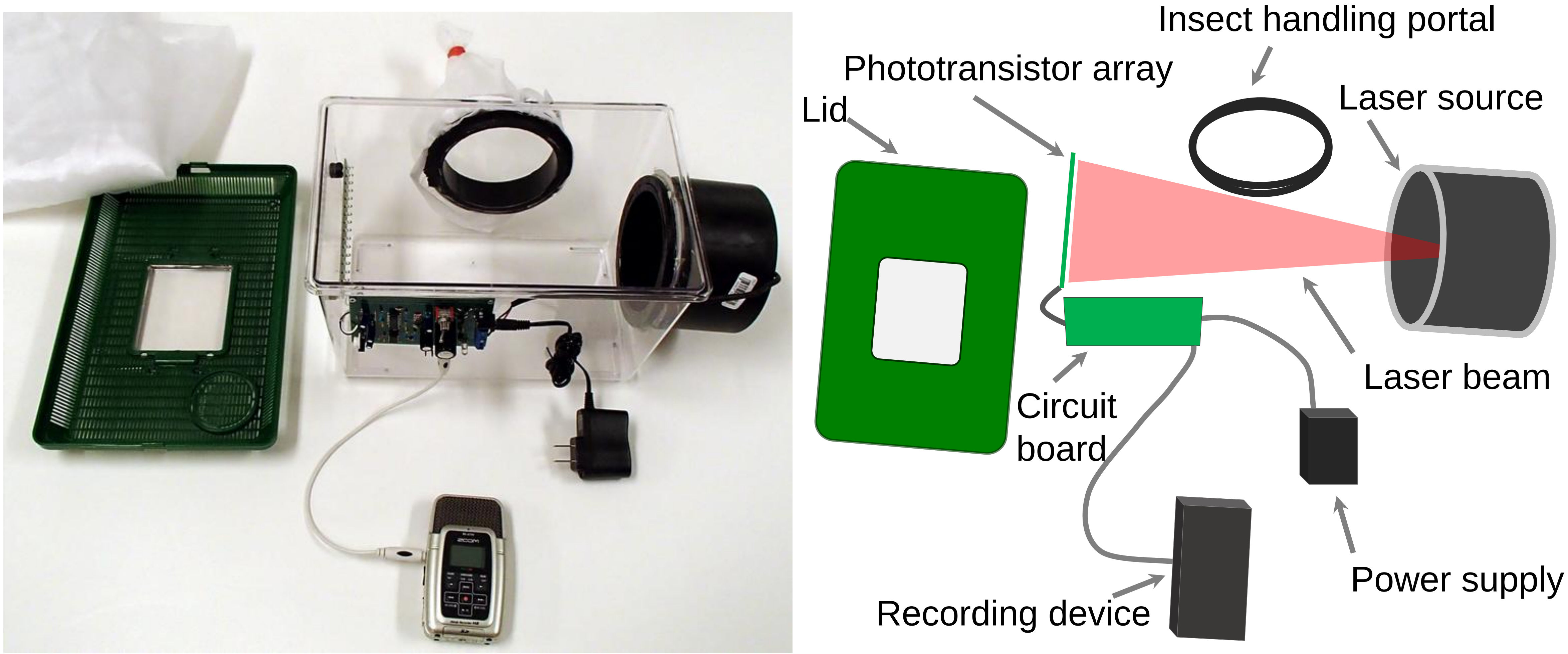

Figure2-sound example

Click here to download Figure: Figure2.pdf

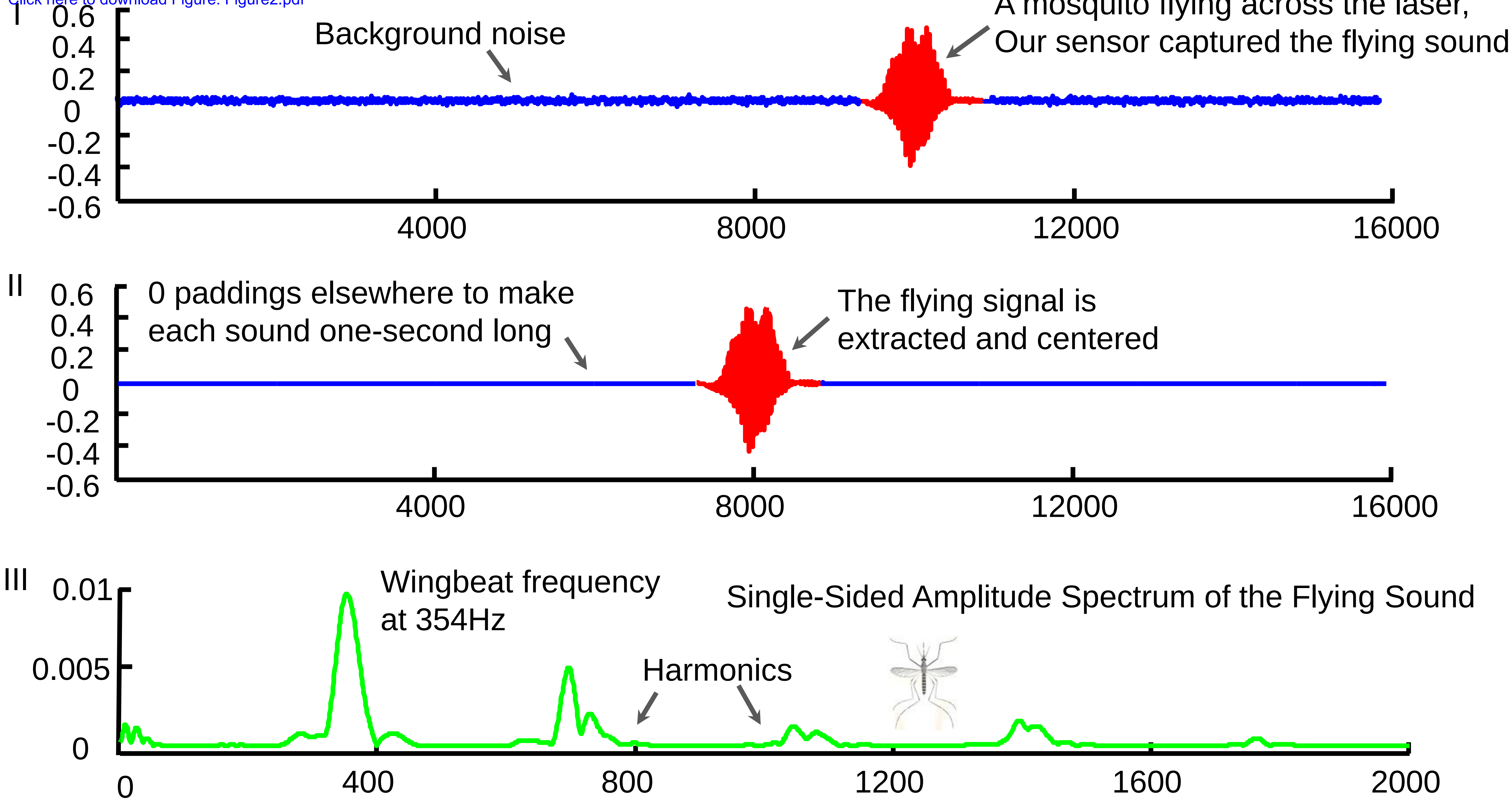

Figure3-Bayesian network with a single feature
Click here to download Figure: Figure3.pdf

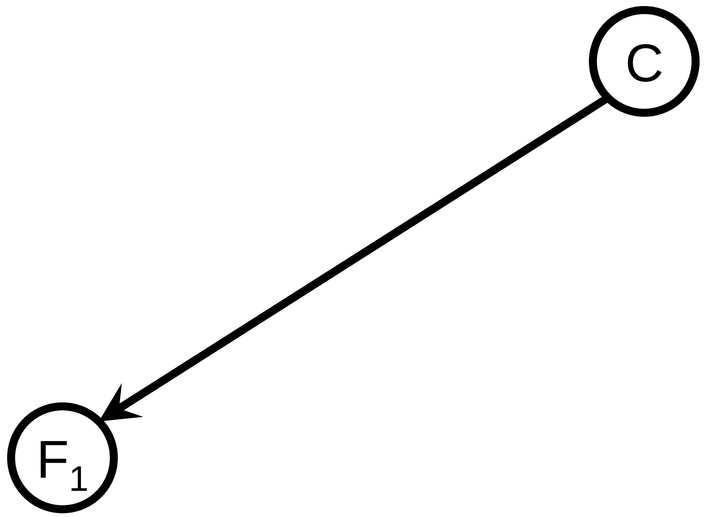

Figure4-Bayesian network with two features

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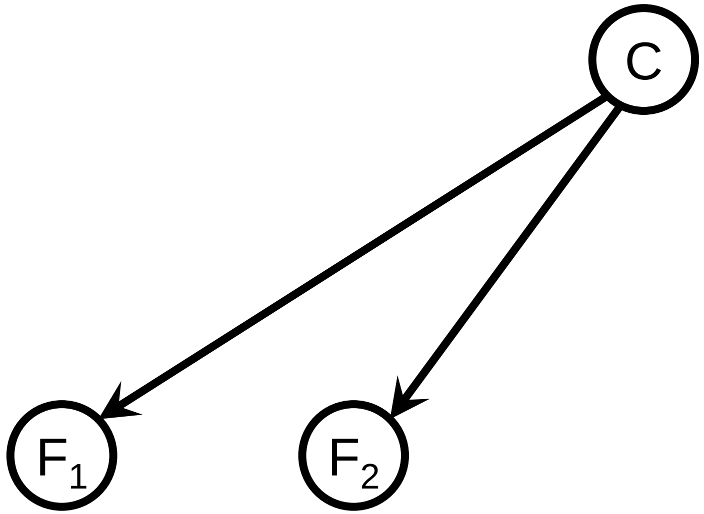

Figure5-flight activity circadian rhythms
Click here to download Figure: Figure5.pdf

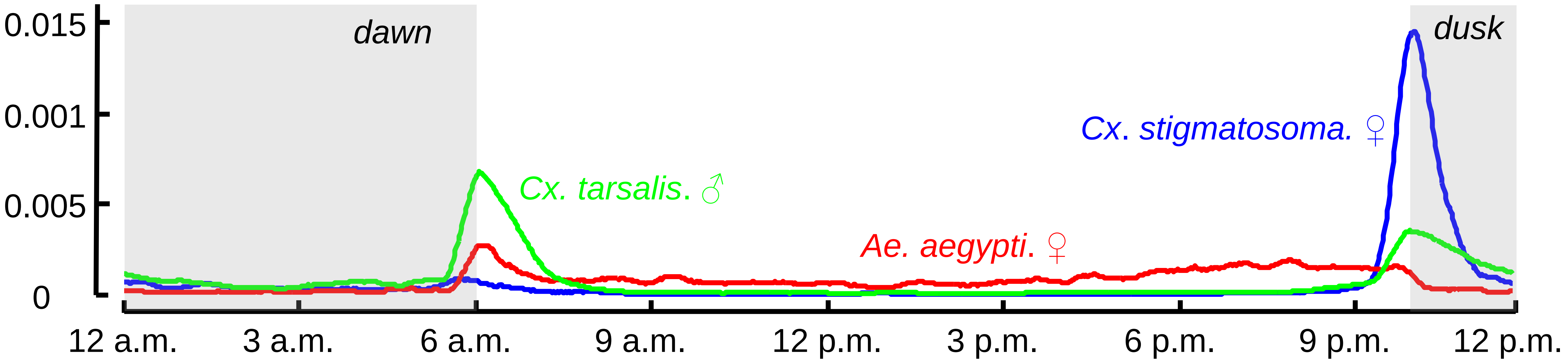

Figure6-Bayesian network with three feature
Click here to download Figure: Figure6.pdf

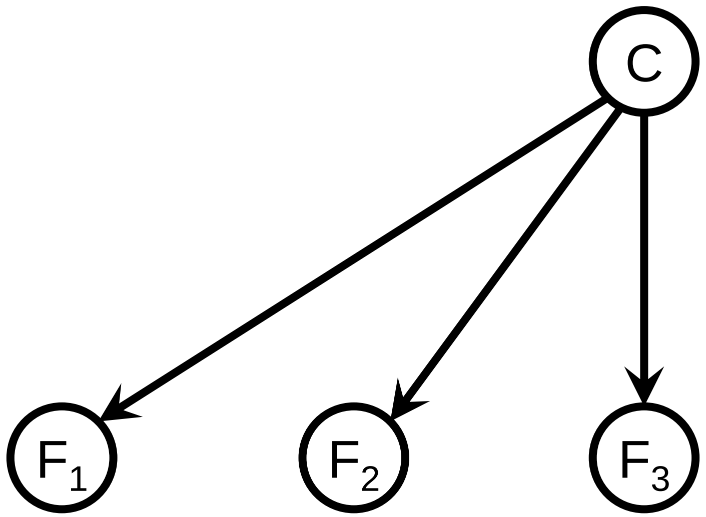

Figure7-geographic distribution

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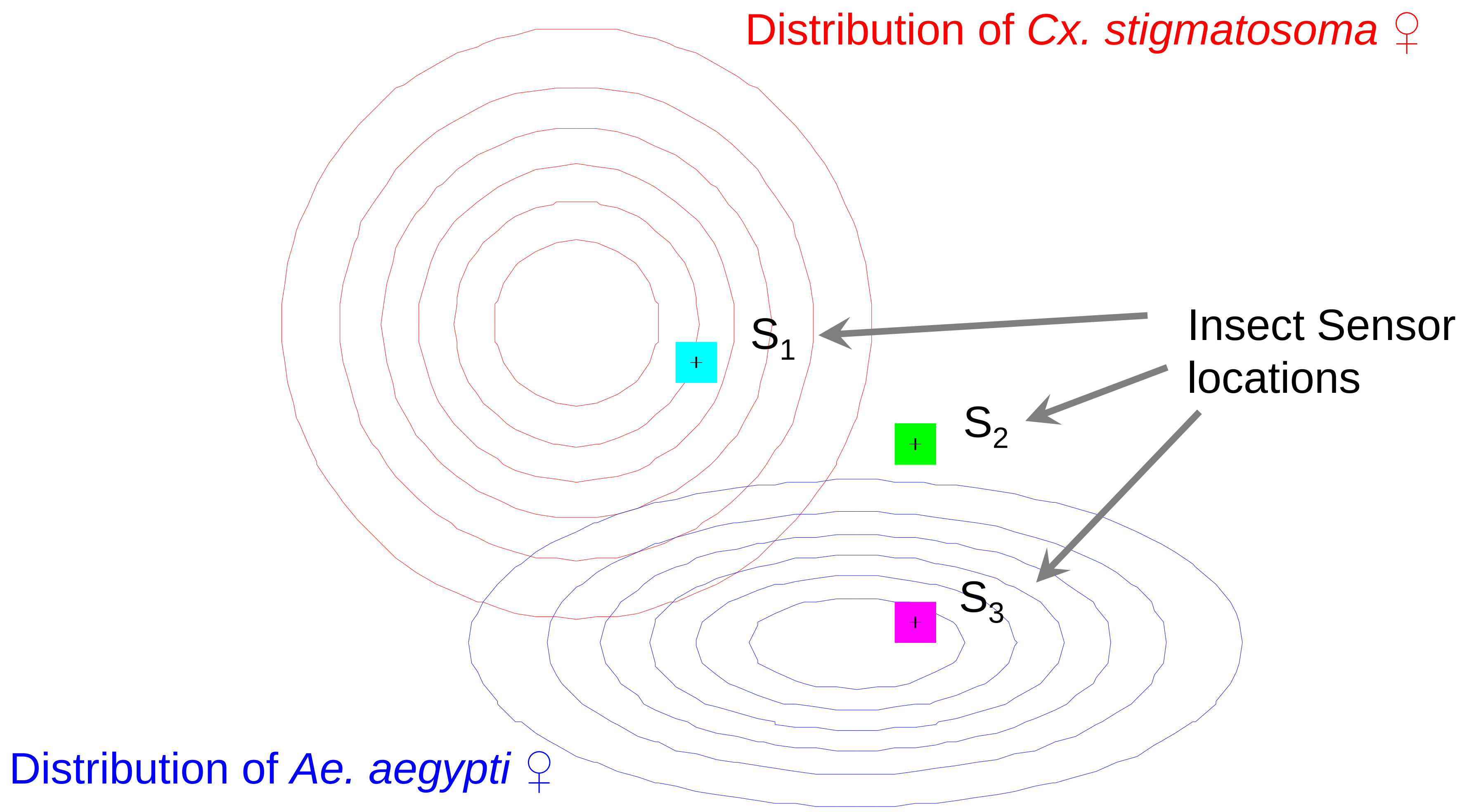

Figure 8-general Bayesian framework
Click here to download Figure: Figure8.pdf

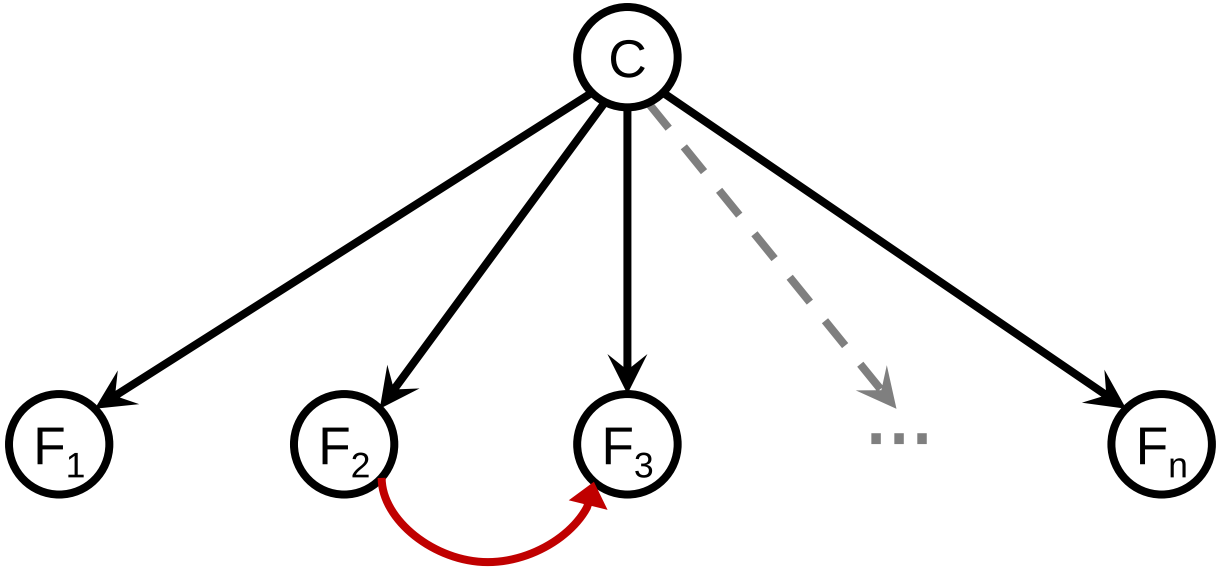

Table2- experiment 2 results

Click here to download Excel Spreadsheet- Table of Materials/Equipment: Table2.xlsx

I (Symmetric cost)		Predicted class	
		female	male
Actual class	female	993	7
	male	5	995

II (Asymmetric cost)		Predicted class	
		female	male
Actual class	female	1,000	0
	male	22	978

Table1- experiment 1 results

Click here to download Excel Spreadsheet- Table of Materials/Equipment: Table1.xlsx

Step	Species Added	Classification Accuracy		Step	Species Added	Classification Accuracy
1	*Ae. aegypti* ♂	N/A		6	*Cx. quinquefasciatus* ♂	92.69%
2	*Musca domestica*	98.99%		7	*Cx. stigmatosoma* ♀	89.66%
3	*Ae. aegypti* ♀	98.27%		8	*Cx. tarsalis* ♂	83.54%
4	*Cx. stigmatosoma* ♂	97.31%		9	*Cx. quinquefasciatus*♀	81.04%
5	*Cx. tarsalis* ♀	96.10%		10	*Drosophila simulans*	79.44%

Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment: CHEN_ JoVE_Materials.xls

Name of Material/ Equipment	Company	Catalog Number
Audio Recorder: ICD-PX312	Sony	4-267-065-11(2)
Insectary	Lee's Aquarium & Pet Products.	20088 HerpHaven®, Large Rectangle
Laser Line Generator, 650nm (red)	Apinex (www.apinex.com)	LN60-650
Photodiode Array	VISHAY SEMICONDUCTOR TEFD4300 PIN PHOTODIODE, 650NM, 20DEG, T1	TEFD4300
Analogue to Digital Convertor Integrated Circuit	Custom made in our lab	

Comments/Description
With a 8 GB microSD extra memory
14 1/2" Long x 8 3/4" Wide x 9 3/4" high. Modified to house insects.
5mW. This is a low powered laser, similar to a teachers lasers pointer
We made a custom array of 15 of these Photodiodes wired in parallel
We made this item ourselves, but an easily available commercial product, Gino PCF8591 AD/DA Converter, provides the same functionality.

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Author(s): Yanping Chen, Adena Why, Gustavo Batista, Agenor Mafra-Neto, Eamonn Keogh

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CORRESPONDING AUTHOR:

Name: Yanping Chen
Department: Computer Science and Engineering
Institution: University of California, Riverside
Article Title: Flying Insect Detection and Classification with Inexpensive Sensors

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Response to editorial comments

Dear Editor,

Many thanks for your detailed comments. We have carefully edited the paper to reflect your suggestions (the newly edited text are in blue). We have addressed your comments in red below for clarity.

1.Your current long abstract is under our 150 - 300 word limit; please modify your long abstract in both the manuscript document and your editorial manager account.
The long abstract has been expanded to be within the word limit.

2.Please remove the bullet format from the introduction section and please replace it with paragraph format.
The bullet format has been changed to paragraph format.

3.Unfortunately there are a few sections of your manuscript that show significant overlap with your previously published work. These sections include your, first paragraph of the introduction. Please re-write that text to avoid plagiarism (including self-plagiarism). We understand that there may be a limited number of ways to describe a complex technique such as yours, but you must use original language throughout the manuscript.

The overlap has been removed.

4.Please re-write steps of your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). For instance, in step 1.1. you mention, “Adult mosquitoes were reared from lab colonies..” should be written as “Rear adult mosquitoes from lab colonies..” similarly in step 1.1.2. Another example, “The experimental chambers were maintained on..” should be, “Maintain experimental chambers..” Please make sure you maintain this imperative style of writing throughout the protocol section.

All the steps have been re-written in imperative tense.

5.Please write the paragraph below step 2.1 in a stepwise manner similar to the protocol section. Non-action items steps can be included as a note.

All steps in the Protocol section have been re-written in a stepwise manner.

6.Please remove commands similar to “[Place Figure 1 here]”, throughout the manuscript.

Commands have been removed.

7.Please state the figure legends below the “Representative results” section. For instance, you mention Figure legends in the protocol.

All figure legends have been removed to below the “Representative results” section, only pointers to the figures (such as, as shown in figure 2) are left in the protocol section.

8.Please make sure to highlight complete sentences. For instance in step 4.1.4.

All the un-complete sentences in the steps have been re-written to be complete.

9.In step 4.1.1, please remove “To compute the sound feature from an insect sound:” and please begin from step 4.1.1.1. Additionally, please consider combining step 4.1.1.1 and 4.1.1.2.

Incomplete sentence has been removed and re-written into the new step. Steps 4.1.1.1 and 4.1.1.2 have been combined.

10.Please try to avoid usage of phrases such as “should be”, “could be”, “would be” and write in the active/imperative style. For example, step 4.1.3.3, step 4.3 etc.

All “should be”, “could be”, “would be” have been re-written into the active style.

11.Please revise the text to avoid the use of any pronouns (i.e. "we", "you", "your", “our” etc.). If you feel it is very important to give a personal example, you may use the royal "we" sparingly and only as a "NOTE:" after the relevant protocol step. Please use the Ctrl+F function to find and replace the pronouns.
All pronouns in the protocol section have been removed, only some are left in sections, such as abstract, introduction and discussion. We have tried to minimize the use.

12.After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is no page limit for the protocol text, but there is a 3 pages limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages (or less) of text to identify which portions of the protocol are most important to include in the video; i.e. which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

We have highlighted the most important 2.75 pages in yellow.

13.Please remove the embedded figure legends from the protocol section. Figure legends, should remain within the manuscript text, directly below the Representative Results text.

The figure legends have been removed to blow the Representative Results text.

14.Please make sure that the “Discussion” section covers the following points running between 3 – 6 paragraphs.
a)Critical steps within the protocol
b)Modifications and troubleshooting
c)Limitations of the technique
d)Significance of the technique with respect to existing/alternative methods
e)Future applications or directions after mastering this technique

Text has been added to the discussion to include all the points listed above, except for the d), which has already been discussed in the introduction session.

15.Please make sure that your references comply with JoVE instructions for authors. In-text formatting: corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text of the manuscript. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume**(Issue), FirstPage – LastPage, doi:DOI, (YEAR).]

We have changed to format of the reference as required, and list them in the order of their appearance in the text.

Rebuttal to reviewer's Comments
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Dear Editor and Reviewers:
Many thanks for your detailed feedback on our paper. We believe that we have addressed 100% of your reservations and questions. For clarity, our responses are in blue text, and all changes made in the paper are also highlighted in blue.
Once again, many thanks for your service to the community.

Editorial comments:
A) The Protocol requires additional detail in several areas:
1)The system set up in step 2.1 should be described in more detail. A table of equipment used could be referenced, but the components should be briefly described in general terms.

2)Please describe how the photoarray is arranged in step 2.1.2, rough dimensions of setup, spacing of lasers, ect.

3)4.1.1.1. How is the DFT used? Is this in a program? What steps are taken to accomplish this?

4)In step 4.1.3.3. How is the posterior probability calculated?

5)How is step 4.2.2.1.1. completed? Is there an equation used?

6)Please verify that matlab code required to run the circadian.wbf file is available in the Chen 2013 reference or included in the supplementary files.

7)Please define the "Bayesian classifier" in step 4.1.3

B)Grammar: "Classify an unknown insect sounds"...

C)The length is at 2.75 pages, but asks in step 4.2.2.1.2 to follow the steps in a section that is not highlighted. This will exceed the length limit unless something else is removed.

D)Visualization issue: In step 4.3.1 "Learn the geographic distribution of insects" doesn't really have a visual. Could the authors suggest something for this?

E)Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

F)Please disregard the comment below if all of your figures are original. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

The Editor’s comments have already been addressed previously. The only change we made is the adding of a note paragraph explaining what a “Bayesian Classifier” is. Many thanks.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The goal of this study is to introduce an inexpensive, non-invasive recording system that could accurately classify flying insects with respect to species and sex, even in a mixed population.

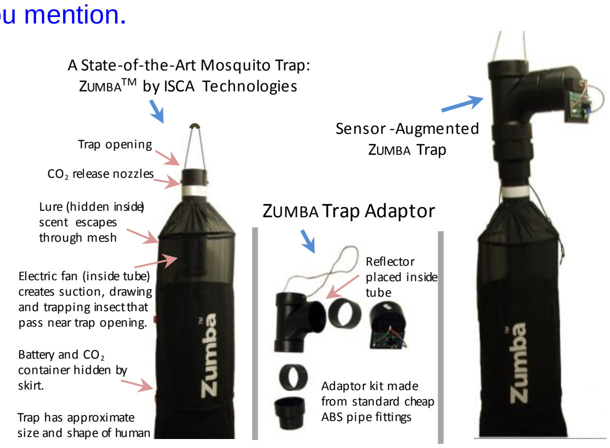
Although the authors tested this system in laboratory setting, they propose that the system will be valuable if deployed in the field for entomological research and medical entomology, e.g. to count and classify insect vectors and assess sex ratios when insect sterile technique is used for population control. The device that the authors have developed show good performance in laboratory setting and is quite effective in classifying many different species of insects, including *Drosophila simulans*, *Musca domestica*, as well as multiple *Culex* and *Aedes* species. The topic of investigation fits of the scope of this journal and this method will be of interest to scientists in other institutions.
Many thanks for your kind words!

However, the reviewer feels that the authors need to address some important issues elaborated below, before this manuscript can be accepted.

The reviewer therefore recommends major revision before this study can be considered for publication. Specific comments that needs to be addressed are detailed below:

Major Concerns:
(1) It was mentioned that these optical sensors can be used to record the "sound" of insect flight from meters away (line 86) with complete invariance to wind noise and ambient sounds - which is great. However, it was also mentioned in lines 411-413 that flashing lights, camera flashes and vibrations near the cages will introduce noise to the data, making it necessary to perform these experiments in the dark room and in places where there is no vibration. I see a big problem with this especially if the goal is to deploy these sensors in the field. I am sure there will be lots of lights and vibrations in the field - does that mean it is not realistic to use this system in the field? I think the authors need to address this. Have they tried this system outdoors (or in a caged outdoor setting) and monitor the quality of the data (i.e. noise level) and accuracy y as compared to lab setting?

We report only on the *lab setting* of our work for brevity and clarity. However we have already considered and addressed all these issues you mention.



First, "*flashing lights, camera flashes and vibrations near the cages will introduce noise to the data,"*. We have design our sensor to be "Lego-like", so that it can quickly be adapted to fit into existing insect traps (of which there are dozens of designs). In the figure to the right we show one such example of our sensor placed in a popular commercial trap called the **Zumba Trap**. Note that here the sensor is inside a darkened ABS pipe, so it is completely "blind" to any external light. Note also that this trap is designed to be suspended, isolating it from vibration. Our sensor *can* sense low frequency movement from wind, or a mouse climbing on the trap, but our software simply filters this information away.
In general, the reviewer is correct in thinking that there are some issues with field deployment. However co-author, Dr. Agenor Mafra-Neto has decades of experience in field deployment of insect traps on six continents, and has been awarded multiple patents for insect traps/counting devices. He has done preliminary field tests with the sensor, it works beautifully in the field.
Our paper simply reflects our understating of the spirit of "journal of visualized *experiments*", not "journal of visualized *deployments*".

(2) If these systems cannot be deployed into the field, then the authors need to address the value

of using them in laboratory setting. It will not likely be used for species identification in lab setting as researchers generally work with known species. What experiments can these sensors be potentially used for? I think the authors have presented really great data, e.g. Figure 5, and illustrated the accuracy that can be attained using their system, but they have to address whether their device can really be used in the field (which is not a darkroom), and if not, then what can researchers use these recording devices in the lab. Maybe they can elaborate?

Please see the answer above, but we will briefly elaborate.

Even without using a physical trap apparatus to isolate our device from vibration and light "pollution", our device still works in the field. The figure to the right shows how we are using the device to study mosquito breeding grounds.

Of course the tree "vibrates" in the wind, but simple signal processing lets use ignore this.

Also, the *shadow* of leaves blowing in the wind would produce a signal if the shadow crosses the photodiodes, but that signal would be 3 to 20Hz. Even the slowest butterflies will flap their wings at 50Hz or greater.

In general, outdoor deployment is *easier* than indoor deployment. Indoors (especially if not on the ground floor), the floor can vibrate if a large person walks past the device. More importantly, indoors certain kinds of cheap fluorescent lights or old style computer/TV screens (CRTs) can produce a lot of optical noise.

In summary, the devices do work well outdoors, however this paper focuses on their indoor deployment.

Phytotelmata (small water bodies held by terrestrial plants) are an important breeding ground for many insect vectors. Here a technician is placing one of our sensors over a water-filled tree hollow. The sensor can be left unattended for up to two weeks on battery power.

Minor Concerns:

(1) Based on the results presented here, the device seemed to produce reliable classification in laboratory setting. But there is always inertia in using new technologies. In order for researchers to adopt this device, the authors need to provide more information about time and cost requirements. In section 3.1 and 3.2, the authors described the method for their detection algorithm and data processing for each 0.1 second long sliding window. To process 3 days of data (which is what they showed in section 2.2.2), how much time is necessary to actually process the data if the researcher has to process 0.1 second at a time. Can they give an estimate? Is it a feasible amount of time?

Done. We have added a note paragraph to explain the time cost. The detection algorithm is much faster than real-time. It takes less than 3 hours (typically around 2.5 hours) to process 3 days data on a standard machine with 2GHz CPU and 8GB RAM. Thanks.

(2) Another aspect that new users will consider is the cost. In line 395 - the authors mentioned inexpensive and scalable. They should provide the estimate cost of building one of these system.

Done. We added the text to explain the cost. The sensor prototype is made of LEGOs, a 99-cent laser pointer, and part of a TV remote, so a set up could be manufactured for less than $10.

(3) The manuscript needs to be edited for spelling mistakes, etc. There is one even in the first sentence of the abstract (line 55, 75, 82, 91).
Done. We also made a pass through the paper to check for spelling mistakes. Thanks.

Additional Comments to Authors:
(1) The feature of adding additional "classifier" is great and especially exciting.
Thanks for your kind words.

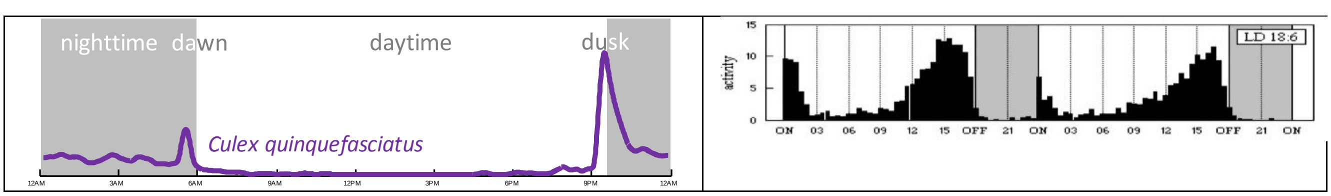
Reviewer #2:
Manuscript Summary:
Fascinating use of optical approach for detecting insects in the field. Thanks!

Major Concerns:
Grandiose in places where it doesn't need to be: "vastly superior" (line 60) vs "superior" or critique of microphones (line 75, 76) "sparse, low-1quality data" vs "have noise rejection challenges" or similar. And then "this work demonstrates that these problems have been solved." vs "Major steps towards addressing these challenges are solved with our approaches." Thanks!

- We have changed : "vastly superior" to “superior”
- As regards “critique of microphones”, we do say “*Such devices are extremely sensitive to wind noise and to ambient noise in the environment, resulting in very sparse and low-quality data*”. However these words are just slightly paraphrased from the original authors that used microphones to record insects. We are just reporting their words.
- We have replaced “This work demonstrates that all these problems have been largely solved.” with “This work addresses all three issues.”

Work appears to be based solely on lab-recordings. The claims are that this system is far superior to others (some of which have been tested and developed in field environments), yet this device has not been subject to field testing. It would be good to present the data for what it is.
As noted above, we *have* done some field tests. However we want to have a narrow, tightly focused scope for this work.

Note that if even if we assumed that our sensor ONLY works in the lab, it still would be a *very* useful tool, as it would allow...
The accurate determination of circadian rhythms.
Below *right* is a typical circadian rhythm plot, averaged over a few hundred data points. Below left is our circadian rhythm plot, averaged over about 100 times more data. Our sensor allows us to collect such data two or three orders of magnitude more cheaply than current methods.



Again, even if we assume that our sensor ONLY works in the lab, it can be used for sexing mosquitoes. Dr. Stephen Dobson of the University of Kentucky is currently using our sensor for (in-lab) sex discrimination.

We could write a long description of the five or six projects our sensor IS being used for, and speculation as to the many things it might be used for. However, we are up against tight page limits

Minor Concerns:
"Sanity check" needs to be revised. What specifically was done?
Done. We have added some text to explain what was done. Thanks.

Reviewer #3:
My preference is to call them laser vibrometer sensors than pseudo-acoustic optical sensors. The term pseudo seems a little confusing in this context.

We ran this idea by the optical engineers in our College; they believe that would cause confusion. The term "laser vibrometer" is generally used to mean a "Laser Doppler vibrometer". However our device is not a Laser Doppler vibrometer [a].

[a] http://en.wikipedia.org/wiki/Laser_Doppler_vibrometer

Line 86. the particular problems mentioned have been largely solved, I agree, but there are others associated with the getting the insects to fly in the chamber. The authors should mention that not all insects may be as cooperative as the ones successfully tested.
Of all the insects we tested, they are all cooperative to fly in the chambers. It's true that some big insects may refuse to fly in the confined environment as in the chambers. However, the chambers are designed only for use in the lab to test and demonstrate the ideas. The final goal is to classify insects in the field, where the insects will not be confined, but live in nature, in which case, we will not have the problem of getting insects to fly.

Line 227. I like this section. It is a how-to recipe on to add important circadian rhythm and geographic distribution information to that received from the sound feature comparisons when conducting automated species identification studies. Thanks for your kind words!

Line 265 "flying generated" should be "wingbeat signals generated"
We meant "flying sound generated". The missing word "sound" is now added. Thanks for pointing this out.

Figs. 3, 4, 6, and possibly 8 could be combined into a single figure.
The multiple figures are used to show how to add features incrementally. In the video version, we will use an animation to show this process dynamically. We have added the animation to the supplementary materials. Thanks.

Fig. 5 The problem with this is that the circadian rhythm is based on the laboratory conditions. This may not be the circadian rhythm in nature.
Yes, all the circadian rhythms shown in this work are based on the lab conditions, and may not be the circadian rhythms in nature. However, in this work, we do not claim that we have learned the circadian rhythms in nature, rather, our goal is to show that it is a good feature to improve the classification performance. In nature, insects' circadian rhythms depend on the time of dawn and dusk, but all insects to be classified are typically at the same location, and thus have the same dusk and dawn time. In our lab, we made sure that the lab conditions are the same for all the testing insects, and thus, we believe the classification improvement made by the circadian rhythms learned in the lab approximates well to those learned in nature would do.

Line 413 "dry towers" ? I don't think that is what the authors mean.
The typo is fixed. It should be “dry towels”. Thanks for pointing this out.

My preference would be to include more outside references such as, Potamitis, Ilyas, Classifying insects on the fly, Ecological Informatics (2013), and to move some of the broad claims in the introduction to a section in the discussion.
We have included the suggested references, thanks. However, since the other idea you have is just a ‘preference’ we respectfully ask that we be allowed to decline. It would take a significant effort and the other reviewers might then object. Moreover, we simply prefer the current presentation, which we converged on after reading some of the most highly cited similar papers in JOVE.

Supplemental File (as requested by JoVE)
Click here to download Supplemental File (as requested by JoVE): animation.pptx

Supplemental File (as requested by JoVE)
Click here to download Supplemental File (as requested by JoVE): 52111_R1_SW.docx