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Optical characterization of agricultural pest insects: a methodological study in the spectral and time domains

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Abstract Identification of agricultural pest insects is an important aspect in insect research and agricultural monitoring. We have performed a methodological study of how spectroscopic techniques and wing-beat frequency analysis might provide relevant information. An optical system based on the combination of close-range remote sensing and reflectance spectroscopy was developed to study the optical characteristics of different flying insects, collected in Southern China. The results demonstrate that the combination of wing-beat frequency assessment and reflectance spectral analysis has the potential to successfully differentiate between insect species. Further, studies of spectroscopic characteristics of fixed specimen of insects, also from Central China, showed the possibility of refined agricultural pest identification. Here, in addition to reflectance recordings also laser-induced fluorescence spectra were investigated for all the species of insects under study and found to provide complementary information to optically distinguish insects. In order to prove the practicality of the techniques explored, clearly fieldwork aiming at elucidating the variability of parameters, even within species, must be performed.

1 Introduction

Agricultural pest insects and insect-transmitted infectious diseases such as malaria are of major concern since they cause much damage. On the other hand, pollinators are necessary for many crops. Clearly, effective methods to monitor flying insects are of considerable interest. The present paper presents a methodological study of spectroscopic techniques for insect monitoring, using a combination of methods operating in the spectral and the temporal domains.

With over 1.4 billion inhabitants, crop production is of great importance to China. Growing population and dwindling cultivated areas have always been a contradiction impairing sustainable development. Agricultural pests play another important role limiting the crop production, which leads to a $250 billion annual loss, constituting 16 % of the total global attainable production [1]. To combat pests and keep the production up, pesticides are being employed. However, when resistance of the pests to pesticides develops, additional substances have to be applied and this may cause severe threats to food safety and reduce profits. Further threats are faced with the predatory and parasitic species due to the wide spectrum of toxicity of pesticides [2]. Also, the farmers who work in the fields are exposed to detrimental effects due to the pesticides [3]. Methodology toward pest control varies between species [4]. Unawareness of the relevant species and pest abundance may lead to abuse of pesticides which can make the situation even worse. Thus, it is important to develop techniques useful

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for pest characterization in the field to achieve better food as well as field-worker safety, as well as profitability.

With awareness of the importance of pest management in crop production, much research has been conducted. Light trapping and pheromone trapping are common methods employed to monitor population dynamics of pests of interest. Direct field visual inspection with appropriate sampling tactics produces absolute estimation of population density. The mark-recapture method is developed to estimate insect population density over a wide area. This method involves to mark captured insects and then release, and subsequently count the relative numbers of marked and unmarked individuals among recaptured insects [5]. However, these methods are labor intensive and expensive.

With the rapid development of radar in the last century, insect monitoring radars have been constructed and applied to entomological research. A vertical-looking radar with Zenith-pointing linear-polarized conical-scan (ZLC) configuration is able to automatically separate locusts, ladybirds, lacewings and ground beetles, hemipterans, silver Y moth and diamond black moth and perform routine monitoring of insects migrating at high altitudes [6–9]. However, it is hard to separate the species with similar size, body shape and wing-beat frequency, and trapping may still be needed for the identification.

Other techniques, including digital image processing and acoustic techniques, are also employed in the study of pests. However, they are in an early stage of development for applications in the field [10].

Optical approaches in insect characterization have developed recently. First, laser-based insect monitoring was performed for the specialized case of honey bees, trained to approach the locations of hidden explosive land mines [11, 12]. Recent development demonstrated the possibility of remotely discriminating species using laser-induced fluorescence, in studies employing a mobile laser radar system and using short pulses at 355 nm [13–15]. Quantitatively monitoring of insects with wing-beat frequency extracted using backscattering from a continuous-wave (CW) laser remote sensing system was developed recently [16, 17]. The reflectance spectra could be recorded with a simple remote dark-field spectroscopy system and be used to discriminate between sexes of two damselfly species [18].

The basic aim of our present work was to perform a methodological study on the possibilities to distinguish insect individuals from each other with a combination of wing-beat frequency analysis and spectral characteristics, monitored through reflectance and fluorescence spectra. This may contribute to the developing of optical pest monitoring and early warning in the crop fields.

We employed a measurement system consisting of a telescope, a fiber-coupled compact spectrometer and a photomultiplier tube (PMT). We investigated wing-beat frequencies and performed spectroscopic characterization of a wide range of pests trapped at the University City, Guangzhou, China. Only optical characterization of four kinds of insects will be presented here to show the potential to distinguish insect species from each other using optical methods.

Further, spectroscopic characteristics using the same experimental arrangements were made for three species of common agricultural pests in the Henan Province, China, and here also gender identification possibilities are considered. This study relates to recently performed field experiments on optical remote sensing of flying agricultural pests in the Zhengzhou area [19]. As demonstrated, it can sometimes be difficult to distinguish insects from each other based on their wing-beat signals only, and then spectroscopic characteristics may provide a remedy.

2 Materials and methods

2.1 Insects

Four species of insects trapped at the University City (113.3815°E, 23.0601°S) part of Guangzhou, in the Guangdong Province of China, are shown in Fig. 1.

Since no attention was put on a specific identification of the species studied, but rather the methodology of optical unsupervised discrimination was explored for the Guangdong Province insects, two unknown but superficially

Fig. 1 Four insect species trapped in the University City, Guangzhou

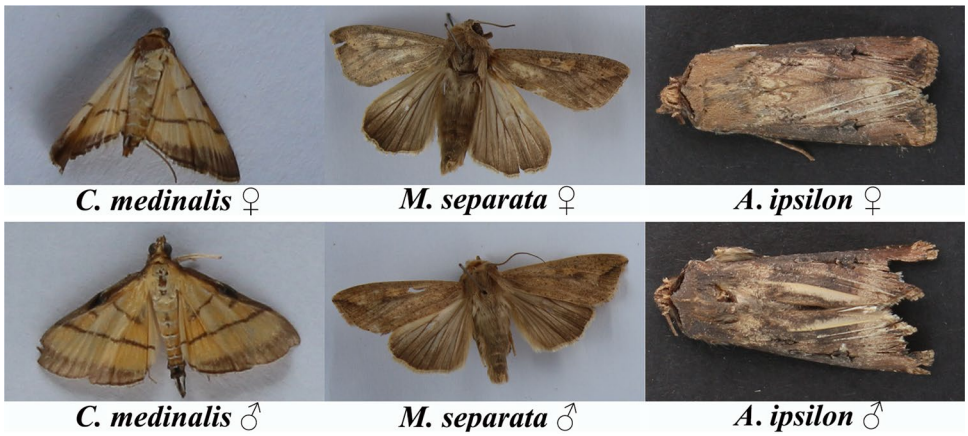

Fig. 2 Three species of common agricultural pests in China, both male and female individuals

visually similar specimens (*I01* and *I02*) were selected, together with two quite different specimens (*I03* and *I04*), where *I04* was, like the samples discussed below, an agricultural insect: Hawaiian beet webworm, *Spoladea recurvalis* (Lepidoptera: Crambidae).

In view of the interest of also being able to distinguish between genders of the same species, related, e.g., to the function of pheromone traps for males, we also performed a spectroscopic study on three biologically well-identified agricultural pests common in the Henan Province as shown in Fig. 2.

The rice leaf roller *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) is a common pest on rice in Asia and Australia. It measures about 16 mm in wingspan. The larva feeds on rice and can cause yield loss up to 50 %.

The oriental armyworm *Mythimna separata* (Lepidoptera: Noctuidae) is a common pest on gramineous crops such as wheat, rice and corn in Asian and Oceanian countries and Pacific islands. It can be found in places such as China, Japan, India, Australia and New Zealand. It reinvades temperate regions every year after having overwintered in warmer or subtropical areas. Its wingspan is 35–50 mm.

The black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae) is a typical noctuid moth which can be found around the world. It is more frequently found in the northern than in the southern hemisphere. Its larva, a serious agricultural pest, feeds on almost all kinds of crops.

The insects studied are mostly dark and brownish in color, with light absorption dominated by melanin, a pigment with strong absorption at short wavelengths and increasingly lower absorption toward the near-infrared region. Thus, reflectance spectra could be expected to be without much structure, with the reflectance steadily increasing toward longer wavelengths in the spectral region studied. Clearly, brightly colored insects are less challenging in identification based on reflectance.

It should be noted that repeated measurements were performed on the same individuals regarding all experiments, i.e., in total only ten individuals were studied. Thus, the data recorded represent the spread observed for each individual. This approach was taken to develop the experimental identification techniques, which was the main purpose of the present paper. As further discussed below, the full usefulness of the techniques would be assessed only in studies on a large number of individuals of the same species where inter-individual variations would also come into play. Such studies remain the subject for future work.

2.2 Laboratory setup for close-range remote sensing of flying released insects

The experimental arrangement is shown in Fig. 3a. We used a black box of size 600 × 600 × 600 mm^3, with two windows of size 200 × 200 mm^2 on both the front and back side of it, to block out surrounding light. A tungsten lamp is placed at one corner of the black box as shown in Fig. 3a. A flat mirror, also of size 600 × 600 mm^2, and somewhat leaning is used to provide more uniform illumination on the released insects. A black termination outside the box is provided by a black paper placed on the laboratory wall. It is important that the FOV of the detection system does not fall on an area of the black paper which is illuminated. The intensity calibration of the system can be achieved by placing a small white polystyrene ball in the window on the back side of the box. It is important to ensure that the detection system operates in the linear regime, since saturation by too high light levels clearly attenuates, e.g., wingbeat modulation.

The basic idea of measurements on flying insect is that a high optical contrast in the light scattered from the insect against a low background can be achieved when an illuminated insect flies into the FOV. When its wings are moving up and down, a periodic change in the optical cross section of the insect will result, as recorded by the

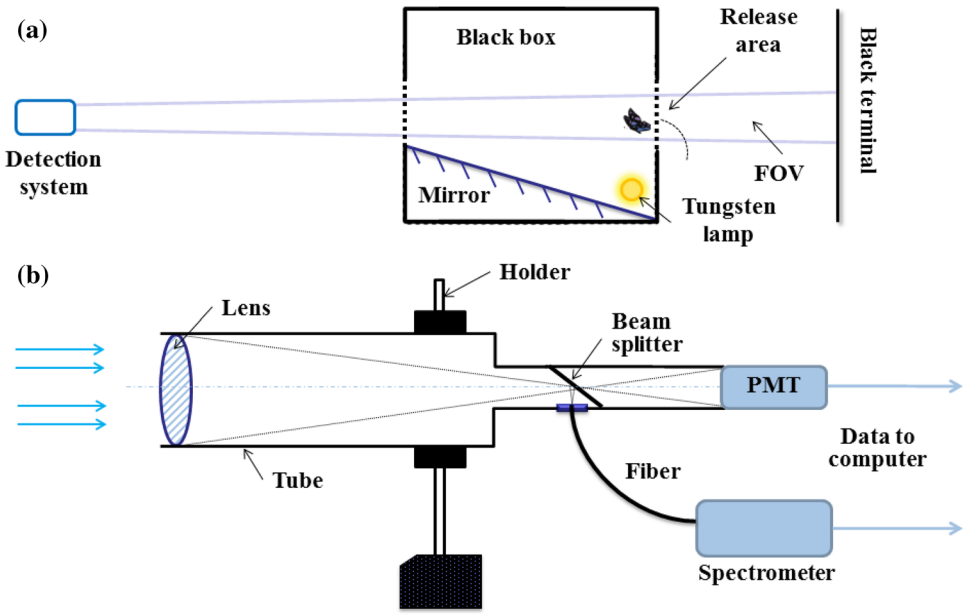

Fig. 3 Schematic diagram of the whole dark-field monitoring system for the study of flying insects (**a**) and details of the detection system (**b**)

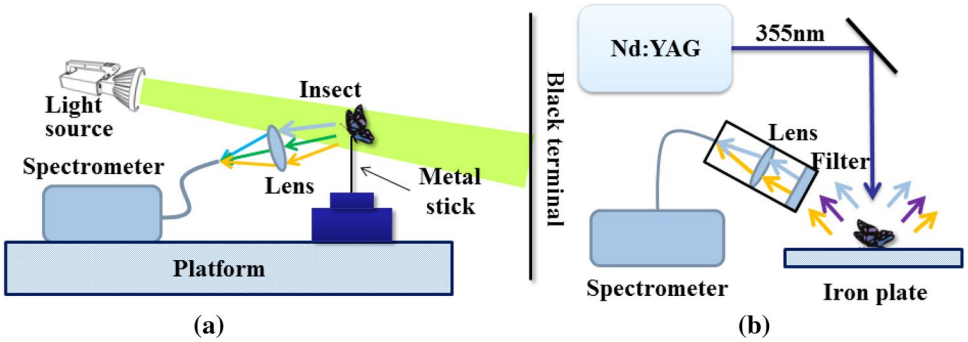

Fig. 4 Experimental arrangements for measuring **a** reflectance and **b** fluorescence spectra

detector. A simple telescope was assembled for collecting the scattered light. A lens with a focal length of 300 mm and diameter of 50 mm was mounted in a metal tube. A 50 % beam splitter was placed symmetrically on the optical axis before the focal point, as shown in Fig. 3b. Two focal points were attained in this way, and the fiber end of a fiber-coupled spectrometer was placed close to one of these two focal points. To achieve a larger detection volume, we actually placed the fiber out of focus. This led to a lower signal compared with the case when placing the fiber exactly at the image plane. To get a spectral signal with sufficient SNR, a 500-W tungsten lamp was employed and a QE65-Pro spectrometer with 1044 detector pixels covering the spectral range 225–1015 nm was used. Compared with the Ocean Optics USB4000 spectrometer, which we used in the field work [19], this spectrometer has ten times lower noise, partly because of active detector cooling. The other light beam after the beam splitter was detected by a photomultiplier tube (PMT; Hamamatsu Model H11526, with a 8-mm-diameter detector) preceded by a neutral density filter (OD = 2) to obtain a light level suitable for the detector. The analog signal was digitized using an USB6009 interface card. Data from the USB6009 as well as the QE65Pro units were transferred to a laptop computer via a USB cable.

While it is relatively easy to get strong PMT signals from flying insects, detection of spectra from the fiber-coupled analyzing unit is more challenging. In order to obtain representative results, each insect was released and recaptured several times till at least five clear spectral signals were observed. As mentioned above, several wing-beat oscillations were recorded by the PMT for each release, because of the extended observational angle of this detector.

2.3 Laboratory setup for measurement of optical characteristics of fixed insect

After our measurements on flying insects, the individual insects were killed and became the subjects for static measurements of spectral characteristics of reflectance and fluorescence, separately for the dorsal and ventral sides. When measuring reflectance, as illustrated in Fig. 4a, the insect

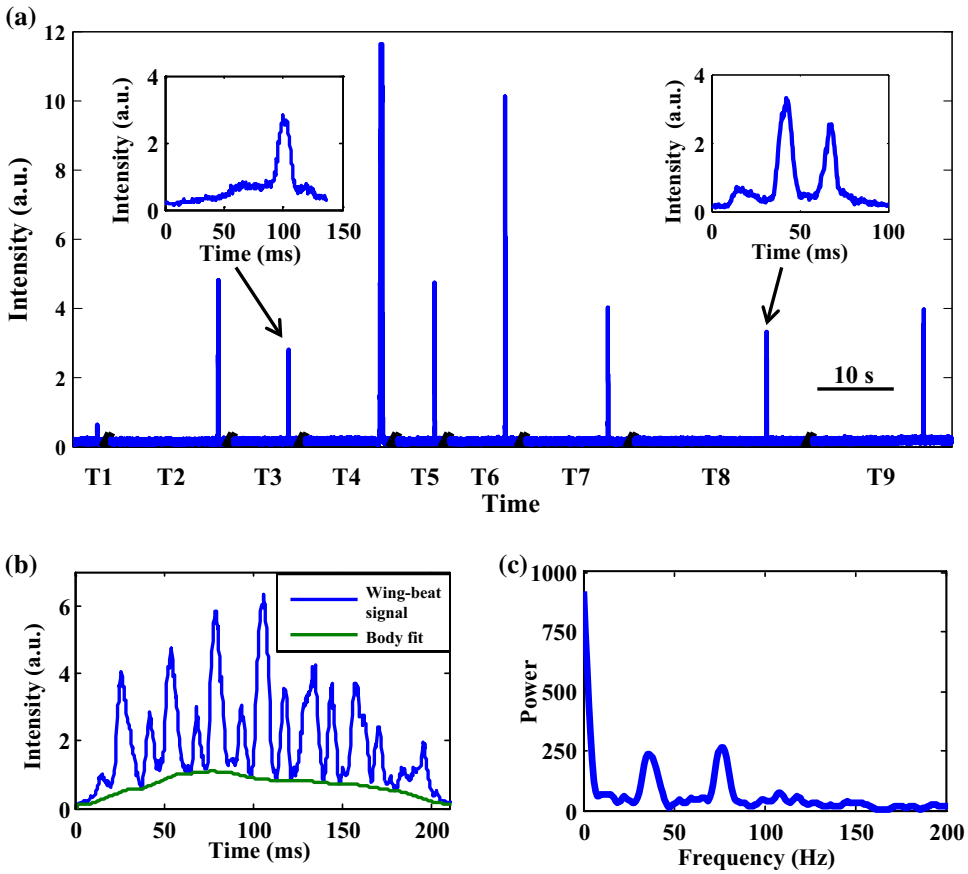

Fig. 5 **a** Recorded backscattering for insect *I01*, repeatedly released into the field of view. Every Ti ($i = 1{:}9$) stands for the time lapse recording for each release. The durations are $T1 = 4.3$ s, $T2 = 16.3$ s, $T3 = 9.1$ s, $T4 = 12$ s, $T5 = 6.33$ s, $T6 = 9.6$ s, $T7 = 14.3$ s, $T8 = 24$ s and $T9 = 19.6$ s. Recapture was conducted at each pause marked with a *black oblique line* in the diagram. The *insets* show expanded views of two of the echoes. **b**. Signal from insect *I03* displaying prominent wing-beats. **c** Fourier transform of the signal shown in **b**

specimen was fixed to the top of a metal pin. An IES-1000 calibration lamp unit was used to illuminate the specimen at an angle, so that no obvious reflection from the black termination would compete with the weak insect signal. Also, a lens was introduced to collect more insect-scattered light. A QE65-Pro spectrometer was again used for spectral recording. When measuring fluorescence as illustrated in Fig. 4b, the insect specimen was placed on an iron plate, which has a very low fluorescence. A Nd:YAG laser was employed to generate the third harmonic (355 nm) for the excitation in the fluorescence measurement. The pulse duration and energy were 10 ns and 1 mJ, respectively. A 400-nm long-pass filter was placed before the fiber to eliminate the strong elastic scattering from the laser. The ambient background was subtracted for each measurement. Given that UV light can be damaging for the pigments and interferometric surface structures of the insect, fluorescence measurements were taken as the last step.

3 Results and discussion

An intermittent, in total 2-min long PMT signal recording of insect *I01* is shown in Fig. 5a, featuring the insect light scattering bursts for in total nine consecutive releases of the same insect, which was repeatedly recaptured. Details of the signal are demonstrated in two smaller diagrams in Fig. 5a. The left one presents only one peak which clearly makes it useless for wing-beat frequency analysis. The right-hand diagram shows only two large peaks and a very small one. Though not an ideal case, it still contains some frequency information although details, like overtone contents, are clearly lacking. Given the fact that *I01* has the largest wingspan among the insects we studied, a low wing-beat frequency is expected. A recording for *I03* is shown in Fig. 5b. The blue curve represents the signal recorded by the PMT, and the green one is the contribution from its body. Subtracting the contribution of the body from the whole signal, we can get the pure signal from the wings, which is more suitable for fast Fourier transformation (FFT). The FFT result of the wing-beat signal is shown in Fig. 5c. With a fundamental frequency of 36 Hz, also the first overtone was strongly present, while the second overtone was faint. The release and recapture procedure was repeated for every insect. We finally got the mean frequency and standard error of each insect individual as displayed in Fig. 6.

Figure 6 shows that the wing-beat frequency varies in the range between 30 and 55 Hz, among which the frequency of *I01* has the largest error bar. A likely reason is

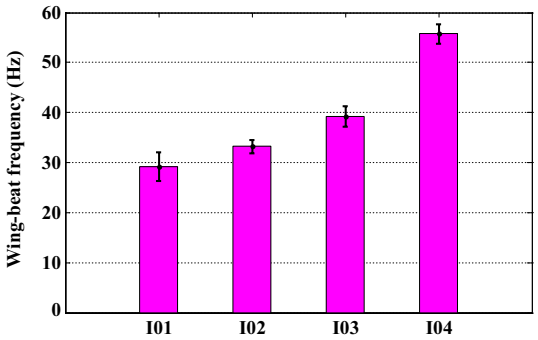

Fig. 6 Wing-beat frequencies of four studied Guangdong Province insects. Standard deviation *error bars* are shown

the relatively fewer number of wing-beat observed in the FOV, as we have discussed before. With the data shown in Fig. 6, we can easily tell *I03* and *I04* apart from the others by observing their wing-beat frequencies. However, distinguishing *I01* from *I02* is observed to be marginal based on wing-beat analysis only. Then, spectroscopic information may improve on the situation.

In the data processing, after deleting spectra with low intensity because of non-optimal passage of the insect through the spectrometer FOV, we got 35 useful spectra for these four species of insects. In order to illustrate the differences among them, we calculated the mean spectral curve for each insect as shown in Fig. 7a. Here, the reflectance is presented corrected to the case of a uniform wavelength sensitivity of the measuring system. A Lambertian $BaSO_4$ reflector was employed. By presenting the individual spectra as points in Fig. 7b, where $F1 = R(980\text{ nm})/R(700\text{ nm})$ is plotted versus $F2 = R(600\text{ nm})/R(490\text{ nm})$, we could easily tell these curves apart and thus the insect individuals. Here, the contrast functions $F1$ and $F2$ were chosen as dimensionless ratios, making them only sensitive to the spectral shape. The spectral classification chosen here was based on the calculation of the slope in different spectral regions. If a third dimension was introduced, we could make a three-dimensional diagram to even separate *I01* from *I04* since the information in the spectra would be utilized in a more complete way. Clearly, a more automatic way to discriminate between different spectra would be to use multivariate analysis [20, 21], where the whole spectral content is utilized and discrimination criteria can be chosen automatically.

To get a better understanding of the spectral characteristics of insects, a further study of the four insects, studied in-flight as discussed above, was performed but now after sacrifice and statically positioned. The main idea behind these additional measurements is to investigate whether there were any differences between static spectra and those captured in flight and how this could be related to flight patterns (Fig. 8).

As we can see, in-flight spectra exhibited a combination of the dorsal and ventral side spectra, while some spectral feature additions were not found in the static spectra. The in-flight spectra were integrations of spectral features from different parts of the insect body exposed during the integration time of 100 ms, long enough to cover several wing-beat periods.

Studies on the morphological structure on the wings of the species Morphidae have shown an evident correlation between the reflectance and incident angles [22]. Analogous research on other Lepidoptera families, such as Noctuidae and Crambidae, whose wings present some interference effects causing iridescence is rather scarce. With further understanding of spectral shapes related to geometry, information in flying direction might be possible from studies of reflectance spectra in flight.

Fluorescence properties of the same insects were also studied, and spectra are shown in Fig. 9. The spectra are corrected for uniform spectral sensitivity employing a standard calibration light source. The fluorescence of insects is very weak and challenging to use in remote sensing. Very weak fluorescence signals were observed for insect *I01* when using an integration time of 1 s. However, stronger signals were observed for

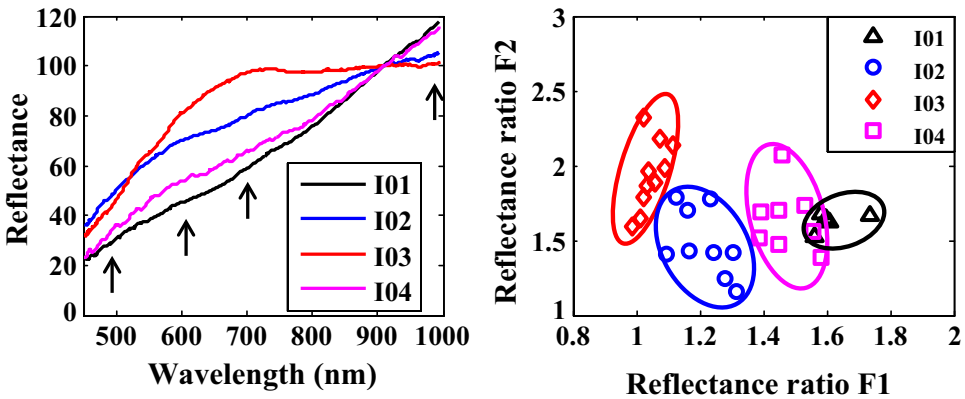

Fig. 7 Mean reflection spectra of flying insects (**a**) and identification between the individuals of different species (**b**). The reflectance is normalized to 100 units at 910 nm for all curves. The contrast functions $F1 = R(980\text{ nm})/R(700\text{ nm})$ and $F2 = R(600\text{ nm})/R(490\text{ nm})$ are employed

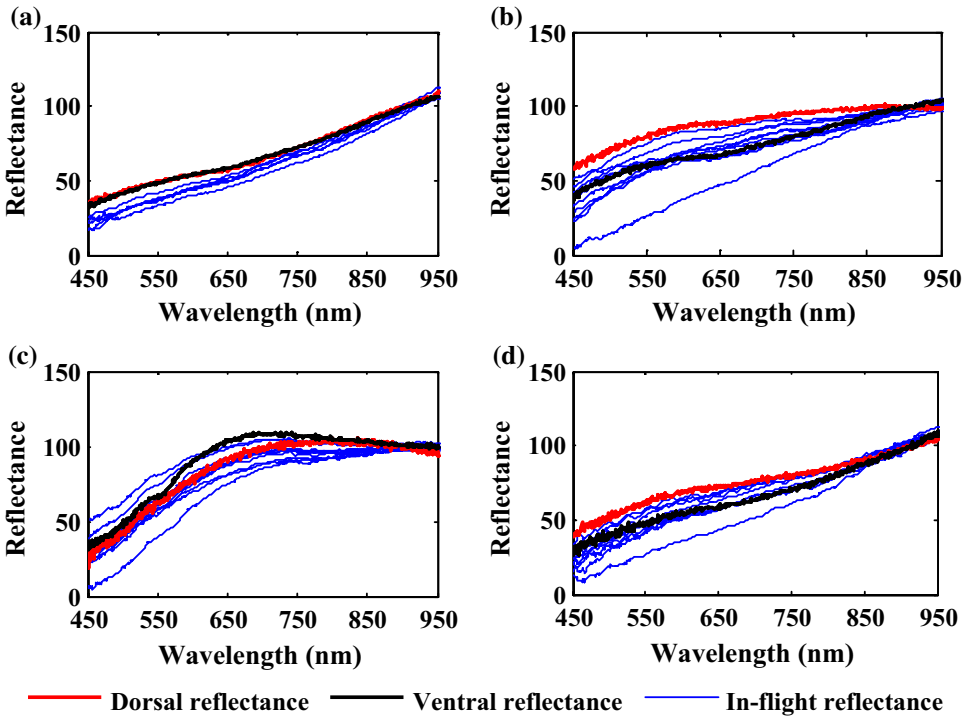

Fig. 8 Static reflectance spectra of insect dorsal (*red curve*) and ventral side (*black curve*), as well as individual reflectance spectra of flying insects (*blue curves*). Data refer to insect *I01* (**a**), *I02* (**b**), *I03* (**c**) and *I04* (**d**). The data have been corrected to a uniform sensitivity for all wavelengths, and the curves are all normalized to 100 units at 910 nm

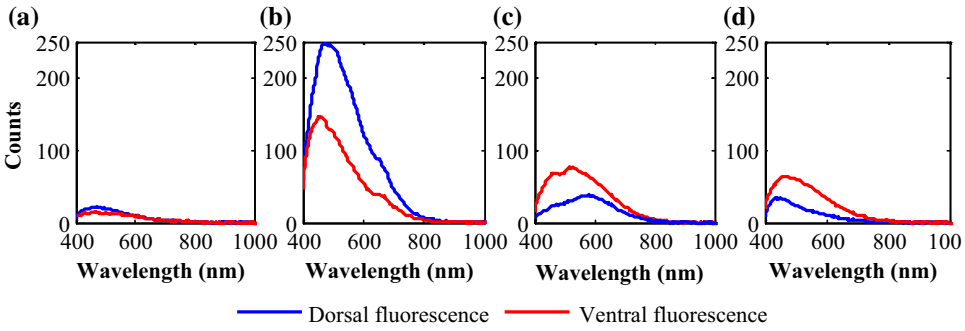

Fig. 9 Fluorescence spectra of insects (**a**) *I01*, (**b**) *I02*, (**c**) *I03* and (**d**) *I04*. The *blue curve* in each diagram stands for signal from the dorsal, while the *red* one refers to the ventral side. The curves are corrected for uniform spectral detection efficiency

insects *I02*, *I03* and *I04*. For each individual, its fluorescence varied between dorsal and ventral sides. *I01* had the most similar shape for both sides with an intensity maximum at 470 nm. The signal intensity of the dorsal side was larger than that of the ventral side. The dorsal fluorescence of *I02* also had the similar shape as the ventral side, but the value was almost twice of that, and the peak at about 475 nm was a bit shifted to the red direction, while the peak of the ventral fluorescence occurred at about 465 nm. Compared with the spectra of *I01*, *I03* and *I04*, a small peak at 650 nm for *I02* on both sides was quite distinguishing. Incidentally, it was noticed that this peak was much enhanced when using an excitation source at 405 nm. *I03* and *I04* had an intermediary signal magnitude, and ventral signals were larger than the dorsal ones, which was quite different from the former two species. A small valley at 490 nm could be seen in the ventral fluorescence of *I03*, while the peak occurred at 520 nm. The dorsal signal of *I03* shifts further to the red region and has its peak value at about 585 nm. In contrast to the insects just described, *I04* has its dorsal fluorescence shifted to the UV direction and peaking at 450 nm, while its ventral fluorescence peaked at 470 nm. All spectra were smoothed with a filter averaging over 10 nm. Studies of fluorescence in the animal world have certainly shown spectacular findings, like the famous green fluorescent protein in jelly fish, awarded by the Nobel Prize in Chemistry in 2008 [23], although all jelly fish certainly do not exhibit much fluorescence [24].

Optical characterization of the three common species of agricultural pests, pertinent to the Henan Province, was also conducted on both male and female individuals. Figure 10 shows reflectance and fluorescence spectra of the dorsal and ventral sides of the individual insects.

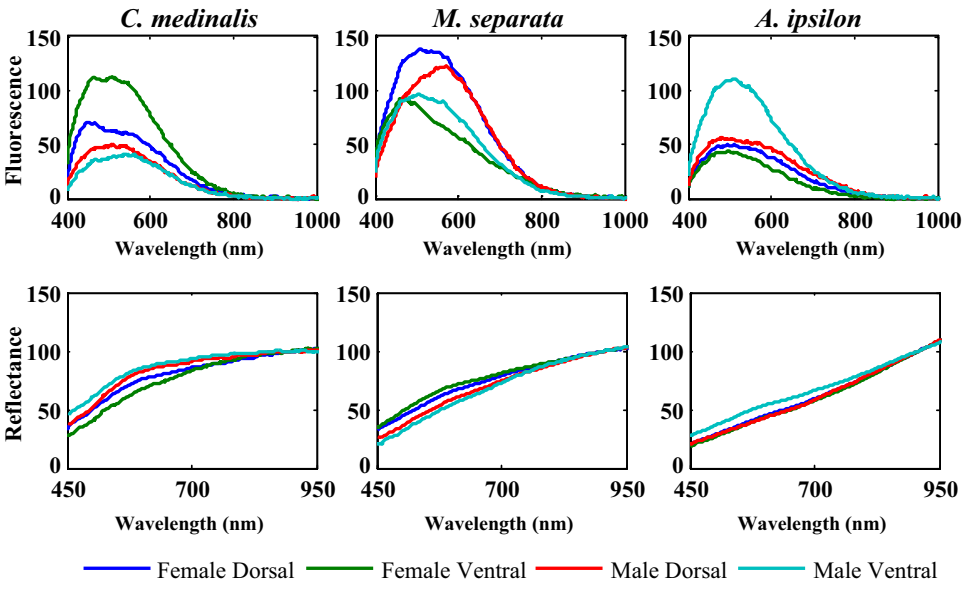

Fig. 10 Dorsal and ventral fluorescence and reflectance spectra of *C. medinalis*, *M. separata* and *A. ipsilon* for both male and female individuals

Though integration time had been extended as long as corresponding to 20 pulses from the Nd:YAG laser, the signals we got are still quite weak. Curves of *C. medinalis* and *A. ipsilon* were quite similar, while a small peak at 460 nm could be seen in the fluorescence of *C. medinalis.* For *M. separata*, the fluorescence of the ventral side peaking at 475 nm shifted toward the UV region compared with the dorsal spectrum peaking at 510 nm for the female and 570 nm for the male.

Although only small differences existed between reflectance spectra of male and female individuals, as well as between the dorsal and ventral sides, we can still tell these three species apart from their reflectance. For *C. medinalis*, the reflectance curve can be divided into two parts, in the first part its reflectance goes up sharper than in the second part, and the turning point is at around 580 nm. The slope of the reflectance curve in *M. separata* is gradually decreasing with increasing wavelength, and it is hard to find an inflection point. For the last kind of insect, *A. ipsilon*, its reflectance curve can be divided into three parts with the slope of the second part apparently lower than the others, while the slope of the third one is larger than the others. Two inflection points are at about 600 nm and 660 nm. We can easily tell these three species apart using these features in the same way as demonstrated above for the Guangdong insects, or with the help of multivariate analysis.

4 Conclusions

We demonstrated the use of an indoor and short-range remote sensing system for optical characterization of flying insects. Advantages of this approach include the possibility to completely control species, temperature and wind speed. The latter parameters are known to influence wing-beat frequencies of insects.

The present study demonstrated the potential of using optical characterization to distinguish pest insect species. Beside wing-beat frequency, the reflectance spectroscopic features worked well to differentiate between the four insect species trapped randomly in Southern China.

The study on specimens of *C. medinalis*, *M. separata* and *A. ipsilon* indicated the possibility of a broader usage of reflectance spectra in discriminating agricultural pests. Fluorescence, although weak in intensity, showed promising potential to discriminate between the two sexes of the same species, as demonstrated in static measurement with 355-nm laser excitation.

It should again be noted that our studies were performed on individual insects. Variation in reflectance and fluorescence spectroscopic features between individuals of the same species was not taken into account in our study, which focused on methodological aspects. However, the present laboratory work, together with our recent fieldwork [19], indicated that it might be possible to use a dark-field spectroscopy system to discriminate insect species and monitor them remotely in the field. Thus, future real-world fieldwork would be highly desirable to elucidate such possibilities.

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