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The potential and prospects of proximal remote sensing of arthropod pests

Christian Nansen*

Abstract

BACKGROUND: Bench-top or proximal remote sensing applications are widely used as part of quality control and machine vision systems in commercial operations. In addition, these technologies are becoming increasingly important in insect systematics and studies of insect physiology and pest management.

RESULTS: This paper provides a review and discussion of how proximal remote sensing may contribute valuable quantitative information regarding identification of species, assessment of insect responses to insecticides, insect host responses to parasitoids and performance of biological control agents.

CONCLUSION: The future role of proximal remote sensing is discussed as an exciting path for novel paths of multidisciplinary research among entomologists and scientists from a wide range of other disciplines, including image processing engineers, medical engineers, research pharmacists and computer scientists.

Keywords: insects; pest management; reflectance profiling; remote sensing

1 INTRODUCTION

The development of the microscope is undoubtedly one of the most significant advancements in terms of technology to perform research, and it is directly linked to at least four Nobel Prizes.[1] The development of microscopes enabled scientists, like Antonj van Leeuwenhoek in the late seventeenth century, to examine and describe insects, protozoans, blood cells and many other microscopic animals and objects.[2] These descriptions led to identification of completely new research questions and to novel hypotheses about cause–effect relationships across all aspects of natural sciences. Furthermore, the initial development of microscopes and the descriptions produced by natural scientists like Leeuwenhoek were unquestionably a great source of inspiration for a wide range of scientists to consider 'microscopy' as a research tool for them to use. Microscopy is essentially a type of 'proximal remote sensing', if remote sensing is defined as 'the measurement or acquisition of information of some property of an object or phenomenon by a recording device that is not in physical or intimate contact with the object or phenomenon under study'[3]. Proximal remote sensing is here defined as acquisition and classification of reflectance or transmittance data with an imaging sensor mounted within a short distance (under 1 m and typically much less) from the target object, such as an insect body. I am in no way arguing that recent developments in proximal remote sensing come even close to matching the importance of the groundbreaking innovations and discoveries by Leeuwenhoek and other microscopists after him. However, there are important similarities in the way the emergence of research technologies (such as the microscope, gas chromatography, qPCR machines, pyrosequencing equipment and advanced proximal remote sensing technologies), reshape the scientific agenda and priorities by enabling us to refine basic research questions and establish new research hypotheses and paradigms. In addition, it is very important to highlight how such technological advances create novel opportunities for multidisciplinary research and teaching.

There are a number of important reasons why proximal remote sensing continues to gain recognition and acceptance as a research tool. Firstly, reflectance or transmittance data are normally acquired non-destructively and within a few seconds, which means that no or negligible preparation of target objects is required. Secondly, the data are digital and therefore quantitative and easy to share via electronic media. The quantitative nature of reflectance or transmittance data means that comparisons normally based on subjective nominal scales (i.e. low, medium, high) can be replaced by much more rigorous thresholds. After purchase of the initial imaging equipment, reflectance or transmittance data can be acquired at fairly low cost, as most proximal remote sensing systems only require small amounts of maintenance, and operating costs are generally low. In addition, the digital nature of reflectance or transmittance data means that they can be acquired in large quantities without major concerns about storage. Although molecular and physiological analyses continue to become less expensive and more powerful, they may still be cost prohibitive at a large scale and require substantial preparation, which is associated with a certain risk of sample contamination. Thirdly, there is the potential of developing processing and classification algorithms to deliver classification results almost

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real time. This may be less important in research applications of proximal remote sensing, but it is probably even more important than sensitivity and accuracy in large commercial operations, such as elimination of objects that are not almonds on a rolling conveyor belt (https://www.youtube.com/watch?v=6Nv2itCkxQ4). The example with almonds on a rolling conveyor belt was included to illustrate a much broader point about the prospects of automated quality control, as the almonds could be almost any type of product and the non-desirable objects being removed from the rolling conveyor belt could be considered 'pests' (individual insects) or food items with pest-induced defects.

Physical conditions affecting the quality of acquired reflectance data include ambient temperature, lighting, projection angle and distance between lens and target object. If these physical conditions can be maintained constant, the fundamental assumption during use of proximal remote sensing technologies is that the radiometric signal (reflectance or transmittance) acquired from objects, such as insect specimens, is determined by their internal temperature, chemical composition and physical structure. Typically, proximal remote sensing data are acquired with high spectral resolution (in hundreds or thousands of narrow spectral bands) and also with high spatial resolution, so that hundreds or thousands of pixels are acquired from a single object (such as an insect body). High spatial resolution of the reflectance or transmittance data being acquired is a very important distinction, because it means that careful radiometric filtering can be deployed as a data processing step to eliminate large proportions of the data and only select a subset with high homogeneity/uniformity data from each object. The main advantage of radiometric filtering is that enhanced data homogeneity increases the likelihood of demonstrating significant between-class separation.

The spectral range of radiometric data being acquired is referred to as 'visible light' when it falls within the visible portion of the radiometric spectrum (between 380 and 700 nm). The visible portion of the radiometric spectrum is divided into six basic light regions: violet (380–430 nm), blue (430–500 nm), green (500–560), yellow (560–600 nm), orange (600–650 nm) and red (650–700 nm). UV light has shorter wavelengths (ultraviolet, 100–380 nm) and the near- and mid-infrared spectra (700–3500 nm) and thermal infrared spectrum (3500–20 000 nm) have longer wavelengths than the visible portion of the radiometric spectrum. Reflectance and transmittance data from all of these portions of the radiometric spectrum may be investigated as part of proximal remote sensing applications, and their performance will be determined by the sensitivity of the sensor and the qualities of the light source. Most of the applications described in this review are based on reflectance data acquired within 400–1000 nm.

It is very important to highlight that spectrometers and imaging sensors have been used extensively for several decades in studies of plant responses to growing conditions and stressors.[4–7] There is also a large body of research studies into the effects of pathogens and herbivorous arthropods on plant health, as described by analyses of plant reflectance profiles.[6,8,9] Similarly, there is a large body of pharmaceutical research into qualitative traits of different types of medical product and their corresponding reflectance profiles.[10–12] Another research discipline with widespread acknowledgement of proximal remote sensing technologies is the food industry as part of studies of food quality and food safety, including (1) quality analysis of meat products,[13–17] (2) detection of mycotoxin-producing strains of *Apergillus flavus* in maize kernels[18] and (3) quality, defects and bruises of fruits and vegetables.[19–22] There are also examples of how proximal remote sensing has been used to detect damage and internal infestation by insects of food products, including field peas (*Phaseolus* spp.),[23,24] wheat kernels (*Triticum aestivum*),[25,26] soy beans (*Glycine max*)[27] and jujubes (*Ziziphus jujuba*).[28,29] These are just a few of the research disciplines in which proximal remote sensing technologies are rapidly becoming mainstream, and they underscore how this technology can be used to detect subtle differences among classes of objects. It also highlights the importance of entomologists with interests in the use of proximal remote sensing to collaborate broadly with colleagues from other disciplines.

Similarly to data mining of molecular data with complicated up- and downregulations, the research challenge associated with analyses of proximal remote sensing data is to identify combinations of specific wavelengths with a consistent response to whatever treatment is being investigated; that is, to identify in which combination of spectral bands there a significant difference between/among (1) two or more species, (2) age classes within a species, (3) males and females, (4) mated and unmated individuals, (5) host individuals with/without parasitism and (6) individuals with/without exposure to a pesticide. These are the types of classification challenge being addressed when proximal remote sensing is applied to studies of insect pests and their management. There are many approaches to analyses of transmittance and reflectance data, including single spectral bands,[30,31] spectral band indices,[32–37] partial least squares,[38–40] principal component analysis,[34,41] linear discriminant analysis,[42] decision trees,[43] neural networks,[44] support vector machines,[8] variogram analysis[23,30,34,35,45–47] and spatial pattern analysis.[48–50] Furthermore, factors such as spatial resolution, spectral resolution, spectral repeatability and penetration depth of reflectance data markedly influence the quality of reflectance and transmittance data and therefore the ability to develop robust and reliable classification algorithms.[51] However, data classification was considered to be beyond the scope of this review, and it was therefore not considered further. Instead, the following provides a review of the current literature on applications of proximal remote sensing in systematics and in studies of physiology and management of insect pests.

2 INSECT SYSTEMATICS AND PROXIMAL REMOTE SENSING

Biosecurity risks associated with invasive pest species is a growing concern in many parts of the world.[52] Furthermore, risks of invasive insect species are increased by factors such as tourism, international trade and climate change.[53,54] Effective responses to protect against invasive pest species include quarantine and inspection policies and procedures that are expensive and time consuming and require technicians with specific training in species identification. Proximal remote sensing can potentially reduce inspection costs and processing time and partially automate some aspects of inspection for invasive insect pest species. Using reflectance data acquired with an RGB camera in three spectral bands (Red Green and Blue portions of the visible light spectrum) of wings and aculeus, three closely related species of fruit flies (*Anastrepha fraterculus*, *A. obliqua* and *A. sororcula Zucchi*) were classified with about 98% accuracy.[43] Although systems based on RGB cameras may not be accurate and applicable for identification of all insect pest species, the concept is intriguing and could potentially be developed further by adding imaging sensors for other portions of the radiometric spectrum and/or imaging sensors with higher spectral resolution.

Based on analyses of hyperspectral reflectance profiles, proximal remote sensing has been used successfully to classify a wide range of insects, including different species of stored-grain insects,[25] two species of fruit flies (*Drosophila melanogaster* and *D. simulans*),[40] tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*),[55] and Klarica *et al.*[38] used imaging spectroscopy to discriminate cryptic species of ants (*Tetramorium caespitum* and *T. impurum*). One of the key potentials of camera-based systems is that they could easily be installed at inspection points, and they may even be connected via the internet to remote supercomputers, which process and classify the reflectance data being acquired at the inspection point. Thus, algorithms installed in a central supercomputer communicating with a large number of camera-based systems could be 'learning' and progressively improving classification capabilities through continuous input and validation (based on molecular analyses and conventional morphology-based species identification) of field samples. The same system could also be used to share digitized models of insects,[56] and thereby reduce the need for shipment of specimens among taxonomists, and to increase the availability of insect reference collections.

Development of imaging-based identification of potential insect pests under quarantine may even be remotely controlled with cameras mounted on robots to inspect imported goods and equipment and a central computer analyzing the data and guiding quarantine officers to particular goods and or pieces of equipment that need further investigation. Based on the video of almonds on rolling conveyor belts, it is even possible to imagine a system in which mass trapping of potential quarantine insects is integrated into the process, and a robotic system is used to sort the insects into individual 'wells' which are subsequently identified on the basis of reflectance or transmittance data acquired by proximal remote sensing technologies. Thus, a robot could subsequently remove individual specimens of certain pest species or food items with perceived pest infestations (based on real-time and non-destructive image classification).

3 INSECT PHYSIOLOGY AND PROXIMAL REMOTE SENSING

Certain portions of the radiometric spectrum, such as X-rays, have shorter wavelengths than UV light and are associated with high energy levels, which enable these wavelengths to penetrate deep into organic tissues. Although X-ray imaging may not conform with the traditional perception of proximal remote sensing, this type of imaging fits the definition of proximal remote sensing used in this review. As an example of basic physiology studies involving advanced imaging, synchrotron small-angle X-ray imaging has been used in a wide range of studies of the physiology and biomechanics of insects.[57] Synchrotron X-ray imaging is considered to be particularly advantageous in studies of minute organisms, and when the objective is to study internal physiological and/or biomechanical responses to treatments. Using an imaging probe, synchrotron X-ray imaging can be used to obtain three-dimensional morphology data with micrometer-range spatial resolutions in fixed and living specimens. This imaging technology has been used to study the respiratory physiology and function of insects.[58,59] In addition, synchrotron X-ray imaging data have been collected *in vivo* from fruit flies to study the changes in thick-filament structure and actin–myosin interactions during flight.[60–62] In another example of advanced imaging, magnetic resonance imaging and magnetic resonance spectroscopy were used to study cold adaptation in larvae of two gall-producing insects, *Epiblema scudderiana* and *Eurosta solidaginis*.[63] The authors developed three-dimensional larval anatomy models and visualized the distribution of liquid water and endogenous cryoprotectants in response to temperature treatments. Importantly, insects subjected to insertion of a synchrotron X-ray imaging probe into the body cavity are alive during imaging but will not survive much beyond the imaging event. Thus, this proximal remote sensing approach cannot be used for data collection from the same individuals at multiple time points during an extended time point.

Phenotypic responses by organisms to treatments and environmental conditions are often complex and challenging to quantify in a repeatable manner. It is important to highlight how many physiological responses by insects are associated with significant changes in the insects' composition of epicuticular hydrocarbons,[64] as they tend to vary (1) among closely related species,[65,66] (2) in relative composition[67] or in actual composition[68] among males and females within a species, (3) among life stages and ages of adults,[66,69–72] (4) among eusocial individuals with different tasks,[73,74] (5) according to mating behavior and status[68,69,75] 6) and in response to environmental conditions.[68,69,76] Owing to the dynamics and complexity of epicuticular hydrocarbon profiles, it seems reasonable to assume that reflectance profiles acquired from insect surfaces may be used to study the basic physiology of insect pests and their responses to treatments and environmental conditions. Although direct correlations between epicuticular hydrocarbon profiles and proximal remote sensing data acquired from the insect body are often lacking, it seems likely that important associations exist and that such associations explain the successful use of proximal remote sensing technologies (1) to age-grade laboratory-reared mosquito species (*Anopheles* spp.)[77,78] and biting midges (*Culicoides sonorensis*),[79] (2) to assess gender, age and presence/absence of *Wolbachia* infection in two species of fruit flies (*Drosophila melanogaster* and *D. simulans*)[40] and (3) to differentiate mated and unmated honeybee queens based on differences in reflectance profiles acquired from the bee abdomen.[80]

4 PEST MANAGEMENT AND PROXIMAL REMOTE SENSING

There are several ways in which proximal remote sensing can be effectively integrated into both basic and applied pest management research. A recent study of adult beetles from two species [maize weevils (*Sitophilus zeamais*) and larger black flour beetles (*Cynaus angustus*)] described how temporal changes in body reflectance were detected in response to two killing agents (entomopathgenic nematodes and an insecticidal plant extract);[81] that is, groups of treated and untreated insects were monitored (via acquisition of reflectance data) over time, and features in their body reflectance were identified, quantified and proposed as indicators of stress to killing agents. The detected changes in body reflectance features occurred after exposure times that coincided with published exposure times and known physiological responses to each killing agent. The results from this reflectance-based study underscore the potential of hyperspectral imaging of the insect body as an approach to quantify non-destructively and non-invasively the insect responses to stress factors, such as stress imposed by exposure to killing agents. To further expand on the potential perspectives of this study, proximal remote sensing technologies may be integrated more broadly

Table 1. Fruit fly species included in the preliminary study[a]

Genus	Subgenus	Group	Species	Origin	Code (Fig. 1)
Drosophila	Sophophora	melanogaster	*suzukii*	Parlier, CA	Swd
Drosophila	Sophophora	melanogaster	*melanogaster*	Catalina Island, CA	Dme
Drosophila	Sophophora	saltans	*sturtevanti*	Wabasso, FL	Dst
Drosophila	Drosophila	repleta	*hydei*	Berkeley, CA	Dh
Drosophila	Drosophila	robusta	*robusta*	Rocky Point, NY	Dr
Drosophila	Drosophila	funebris	*funebris*	Sturgis, KY	Df
Drosophila	Drosophila	immigrans	*immigrans*	San Diego, CA	Di
Drosophila	Drosophila	melanica	*paramelanica*	Muscatine, IA	Dpa
Scaptodrosophila	Scaptodrosophila	victoria	*lebanonensis*	Veyo, UT	Sc

[a] Fruit fly pupae (24–48 h old) were exposed to parasitism by the ectoparasitoid *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae) when the pupae were 48–72 h old. Proximal hyperspectral imaging data were acquired when the pupae were about 96 h old.

Color Figure - Online only

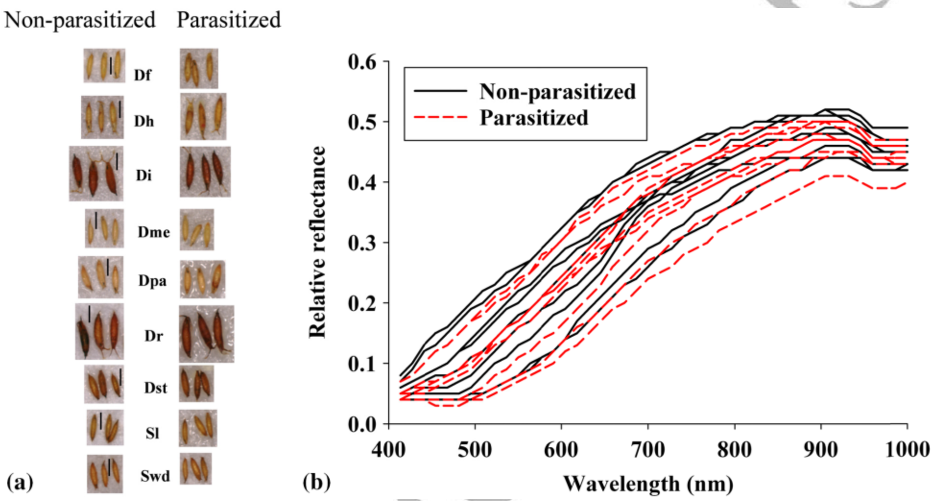

Figure 1. Representative photos of pupae from the nine species of fruit flies with/without parasitism by the ectoparasitoid *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae). The vertical black bar in each photo of non-parasitized pupae represents 2 mm (a). Average relative reflectance profiles from fruit fly pupae with/without parasitism in spectral bands from 400 to 1000 nm (b).

into studies of insect toxicology as part of the characterization of body (or specific tissue) responses in terms of reflectance to target-site and metabolic resistance mechanisms and or sublethal responses to exposure to low insecticide dosages.

Proximal remote sensing may also be integrated into studies of the performance and host selection by parasitoids. Nansen *et al.*[30] demonstrated that three species of juvenile egg parasitoids (*Trichogramma*) developing inside moth host eggs could be accurately classified on the basis of proximal hyperspectral imaging data acquired from the host eggs. This point is illustrated further from acquisition of hyperspectral imaging data (240 narrow spectral bands from 383 to 1036 nm) from pupae (24–48 h old) of nine species of fruit flies (Table 1 and Fig. 1) with/without parasitism by adult females of the ectoparasitic parasitoid *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae), which places eggs on the host surface inside the puparium. The pupae were exposed to parasitoids for 24 h when the pupae were 48–72 h old. After parasitism, pupae were placed inside a petri dish on moist tissue paper, and proximal hyperspectral imaging data were acquired when the pupae were about 96 h old. A total of 180 average reflectance profiles were obtained from individual pupae [nine species × two treatments (with/without parasitism) × ten replications = 180 reflectance profiles]. From the photos of pupae it can be seen that there was more variation in colors among species than between non-parasitized and parasitized pupae within species (Fig. 1a). Average reflectance profiles of parasitized and non-parasitized pupae followed similar general trends but varied considerably in relative reflectance intensity, with parasitized pupae generally being darker (lower reflection) than non-parasitized pupae (Fig. 1b). However, Fig. 1b shows that average reflectance profiles varied considerably among the included species, and that even though reflectance profiles from parasitized pupae were generally darker than from conspecific non-parasitized pupae, there were several parasitized pupae with higher average reflectance (lighter) than non-parasitized pupae from other species. Thus, based on Fig. 1b, it is clear that reflectance in a single spectral band would be insufficient for accurate separation/classification of non-parasitized and parasitized pupae across species. Instead, linear discriminant analysis[82] and data processing steps described in similar studies[23,30,83–85] were used to classify non-parasitized and parasitized pupae. In addition, spectral binning was deployed so that the 220 original spectral bands were averaged into 44 spectral bands to eliminate the risk of overfitting of the classification model.[42,86–88] Of these 44 spectral bands, 26 were used to create a classification model that separated the nine species of non-parasitized pupae with about 88% accuracy. Subsequently, classification models were developed for each of the nine species to separate non-parasitized and parasitized pupae, and all nine models were associated with >95% classification accuracy. This preliminary study therefore demonstrates that, once a robust and sensitive classification algorithm has been developed, hyperspectral imaging can be used effectively to separate closely related fruit fly species based on pupal reflectance features, and also that parasitism in each species is detectable with a very high level of classification accuracy.

5 CONCLUSIONS

In many aspects of pest management, a major constraint is how to acquire and process large quantities of data (in both space and time), and ultimately how to convert big datasets into sustainable and cost-effective pest management solutions. Airborne remote sensing technologies are being integrated in a wide range of crop management practices, including irrigation,[89] fertilization,[90] weed detection,[91,92] yield mapping[93] and pest management.[51] In addition, proximal remote sensing enables acquisition of reflectance and transmittance data with high spectral and spatial resolutions, and this review has demonstrated how this technology is being successfully integrated into different aspects of insect systematics (including insect pest identification) and in studies of insect physiology and biological control. This trend is in itself creating many new opportunities for research and elucidating novel possibilities to investigate hypotheses about cause–effect relationships. For instance, the non-destructive nature of reflectance and transmittance data enables continuous monitoring of the same individuals over time and quantification of how they respond to imposed experimental conditions, including pesticide-treated surfaces.[85] Careful analyses of temporal changes in body reflectance responses acquired non-destructively may be used to optimize timing of physiological and molecular (destructive) interventions to elucidate changes in gene expression and/or changes in biochemical pathways. Using proximal remote sensing technologies, the ability to detect and classify objects with subtle differences in reflectance or transmittance not only opens up new avenues of research, it also creates a very intriguing collaborative platform for software engineers, image analysts, electrical engineers, ecologists, insect physiologists and agronomists to conduct research and teaching into machine learning, machine vision, automated inspection and improved quality control.

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