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In-hive flatbed scanners for non-destructive, long-term monitoring of honey bee brood, pathogens and pests

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ABSTRACT

Honey bee colonies face significant threats from pathogens and pests, including chalkbrood disease caused by *Ascosphaera apis* and *Varroa destructor* mites. Traditional monitoring methods for these issues are often destructive, hindering continuous and detailed observations. This study introduces a novel, non-destructive monitoring technique using a modified flatbed scanner integrated into a honey bee brood frame. The scanner, housed within a Dadant frame and connected to a Raspberry Pi, captures high-resolution images of the brood cells at regular intervals. This method enables continuous observation of the brood life cycle, including egg laying, larval development, and the presence of pathogens and mites. Over a three-month pilot study, the scanner successfully monitored 419 cells, capturing 2819 images of each cell and documenting critical events such as Varroa infestations and chalkbrood development. The method demonstrated high-resolution imaging capabilities, enabling detailed analysis of pathogen dynamics and hygienic behaviors like Varroa-sensitive hygiene (VSH) without apparent disturbance of the colony. The results revealed a high frequency of brood removal and pathogen detection, providing insights into the natural behaviors of honey bees and their interactions with pests.

1. Introduction

The health and functioning of a honey bee colony are intricately tied to the condition of its honey and hive combs. Each comb comprises thousands of cells, utilized for storing nectar and pollen or for rearing brood. However, these cells also provide habitats for various pathogens and pests, including the chalkbrood fungus (*Ascosphaera apis*) and *Varroa destructor* mites, which complicate colony management and health.

Chalkbrood disease, caused by *A. apis*, infects larvae through ingested spores, leading to fungal mycelia growth and the eventual death of the larvae. Infected larvae transform into hard, chalk-like “mummies”, posing a significant challenge to colony health [1]. The disease manifests when larvae are chilled around the capping time of the cells, a condition difficult to replicate accurately in research environments [2,3]. Despite attempts to control chalkbrood by selecting colonies with efficient hygienic behavior, current methodologies often fall short. They either fail to simulate natural conditions accurately or involve invasive techniques that disrupt colony health.

Similarly, Varroa mites represent a major threat to honey bee colonies. The mites’ life cycle is closely tied to the brood, with their reproduction rates often varying significantly between laboratory and field conditions [4–6]. Effective monitoring of Varroa infestations has traditionally relied on labor-intensive and destructive methods, such as manually examining hundreds of cells and pupae [7,8]. These methods can weaken the colony and provide skewed results due to the disturbance caused by cell removal.

Hygienic behavior in *Apis mellifera* bees, including detecting, uncapping, and removing diseased or dead brood, is a crucial component of colony health management. Varroa-sensitive hygiene (VSH) specifically targets Varroa mites by interrupting their reproductive cycle, which can significantly reduce mite populations and improve colony resilience [9]. Despite the benefits of VSH behavior, the ability to study and monitor these behaviors effectively remains limited [10,11]. Existing methods either do not accurately replicate natural conditions or are destructive, undermining their utility for continuous observation [7,12,6].

To address these limitations, we propose an innovative method that integrates a thin flatbed scanner into a brood frame, allowing it to be placed inside a hive. This setup features a 3D-printed, wax-coated mesh foundation on the scanner glass to support brood care. Connected to a Raspberry Pi computer, the scanner captures images of cells at regular intervals, enabling continuous observation of the bee brood lifecycle (eggs, larvae, pupae) and the presence of pathogens, nectar, and pollen.

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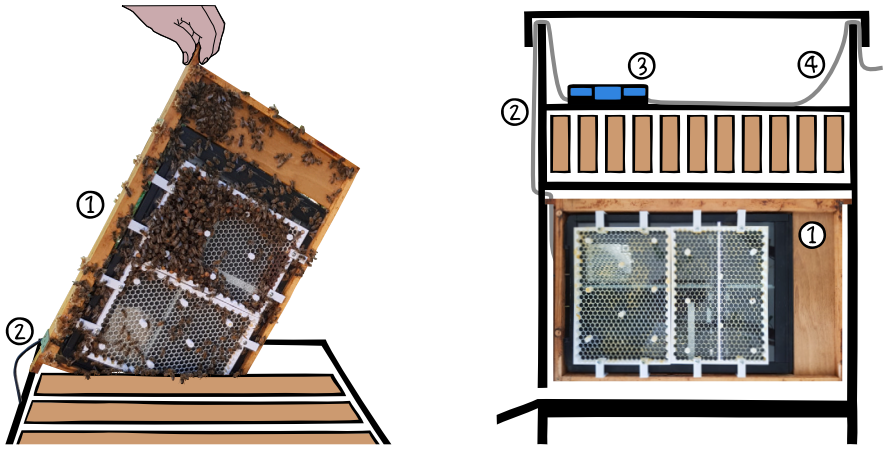

Fig. 1. (a) The scanner (1) is inspected. Note the white plastic foundation, which was coated with a thin layer of wax before its initial insertion. (b) The control unit (3) is connected to a mains power supply (4) and placed in an empty honey super on top of the hive. A single USB cable (2) transfers both power and data between the controller and the scanning device.

This study aims to develop and explore a non-destructive method for monitoring Varroa mites and chalkbrood in honey bee colonies. We hypothesize that the integrated scanner method will:

1. Facilitate continuous, non-destructive monitoring of Varroa infestations and chalkbrood infections.
2. Provide more accurate and detailed data on Varroa and chalkbrood dynamics compared to traditional invasive techniques.
3. Enable observation of hygienic behaviors, such as VSH and general brood removal, without apparent disruption of the colony.

By integrating advanced technology with traditional beekeeping practices, our research addresses significant gaps in the field and provides a valuable tool for enhancing hive health management. This method represents a new perspective on monitoring bee health and pathogen dynamics, potentially leading to more effective strategies for managing honey bee colonies.

2. Materials and methods

2.1. Hardware setup

Scanners designed for A4 paper (210 × 297 mm) fit well into the standard frames of many beehives, requiring no additional protective measures and making installation straightforward. For this study, a CanoScan LiDE 210 consumer scanner was selected due to its wide availability, cost-effectiveness, and thin profile (39 mm, including the lid). It produces high-resolution images up to 2400 dpi, although higher resolutions slow scanning speed. For operation in the hive, the lid was removed, and the scanner was integrated into a Dadant frame (see Fig. 1(a) and (b)). A small cut-out in the brood-box facilitated cable management. The scanner is powered via USB, requiring only a single cable for power and data transmission.

The control unit, a Raspberry Pi 4 Model B, was housed in an empty honey super on top of the hive to protect it from moisture and from the bees. Since a mains connection was available, energy considerations did not influence the choice of the control unit. The Linux-based operating system enabled scheduled tasks to be executed automatically at specific times or intervals. These cron jobs effectively reduce the required software to a single scan image command provided by the `sane-utils` software package.

2.2. Foundation design

To facilitate the construction of transparent-floor cells on a scanning surface, we used a 3D-printed scaffold honeycomb structure that securely clipped onto the scanner. This scaffold provided a stable base and support for the bees, allowing them to build their cells in a controlled and observable environment. Fig. 1(a) shows the brood frame with the built-in scanner and the white plastic foundation. The choice of cell diameter influences whether worker or drone brood is laid. The mesh, designed with OpenSCAD software (https://openscad.org), had an inner cell diameter of 6.9 mm (drone brood) and was made of polylactic acid (PLA). The mesh height was 1.4 mm, which the bees continued to extend. Following standard practice [13], we coated the plastic mesh with a thin layer of liquid wax to enhance its acceptance by the bees.

2.3. Pilot study

The study aimed to demonstrate the feasibility of using a flatbed scanner within a bee colony. Image data were collected at 30-minute intervals over three months, starting from April 13, 2024 to July 10, 2024. The study used a queen-right honey bee colony located in Karlsruhe, Germany, housed in a Dadant brood box.

Given drone brood's significant role in Varroa reproduction and the high probability of finding infested cells (97 %, see Odemer et al. [14]), we targeted this brood type using drone cell foundations on the scanner. In western honey bees, drone brood infestation is approximately ten times higher than that of worker brood [15–17]. This preference is attributed to several factors: drone development extends by two additional days, providing mites with more time to reproduce [16]; the pre-capping period during which drone brood attracts mites is two to three times longer than that for worker brood [7]; drone brood is more frequently visited by nurse bees, increasing the likelihood of mite transfer [18]; and drone larvae produce higher levels of kairomones, which attract mites [19].

Following common practice, the drone frame was first placed on the outer edge of the hive. After one week, the frame was repositioned closer to the center of the brood nest to improve acceptance. However, the choice of brood type can vary depending on the study; in some experimental setups, a foundation with worker or mixed brood might be more suitable.

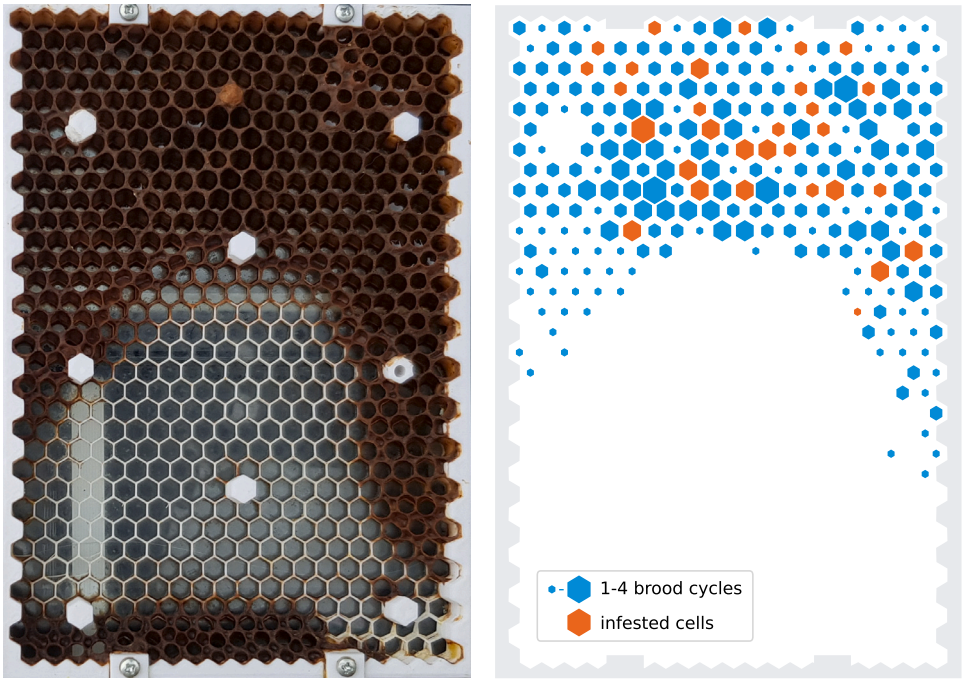

Fig. 2. (a) The drawn out portion of the scanner at the end of the 3-month period. (b) The total number of brood (hatched and removed) per cell, with the size indicating the brood count ranging from 1 to 4. Cells where Varroa mites were detected at any point are highlighted in red. No cell experienced more than one invasion.

2.4. Image acquisition and annotation

Initially, images were manually triggered until automation began three days after the first egg was laid (May 8, 2024). A custom annotation tool was developed to (1) extract individual cells (768 × 768 pixels) from the large source images (20 464 × 28 110 pixels) and (2) facilitate rapid iteration and labeling of these clipped images. Labels included egg laying, bee bread, adult Varroa (including position), Varroa offspring (including position), brood removal, hatched bee, and chalkbrood onset.

2.5. Data management

At 2400 dpi, image acquisition takes around 10 minutes per scan, producing images of 30-40 MB each, amounting to approximately 1.6 GB of data per day. A WiFi connection was used to transfer data from the Raspberry Pi's SD card, preventing storage issues on the device. Given that images of this size are time-consuming to load, center-cropped cells were automatically extracted and organized into folders, allowing for quick navigation and inspection. Efficient data management strategies are essential to handle the large volume of information generated during the continuous monitoring period, especially when multiple devices are used in parallel.

3. Results

3.1. Hive environment and behavioral observations

Unfavorable weather at the study's start hindered comb building. After moving the scanner closer to the brood nest, bees began drawing out the foundation, with the first eggs observed on May 8, 2024. The wax layer was observed to be repurposed for comb building, expanding over time but covering only about one-third of the scanner surface. Fig. 2(a) shows the state of the drawn out portion of the comb at the end of the trial while Fig. 2(b) shows the occupation and infestation of each cell spatially aligned.

3.2. Image data collection and annotation

During the study period, a total of 2819 images were recorded and manually labeled for each of the 419 cells. Larvae and pupae generally show limited movement during most stages of their development, resulting in minimal changes between images. Consequently, significant events, such as the appearance of Varroa mites, the development of chalkbrood, or the removal of brood, are well detectable even in rapid iteration through all the 1 181 161 relevant cell images. Animations of particularly notable cells are available in the supplementary material.

Due to its size of over 100 GB, the raw image data will be made public only upon request. The annotations can be found in JSON format in the supplementary material.

3.3. Brood development

A total of 511 eggs were laid, with approximately 58.32 % of these being removed before hatching. Fig. 3 illustrates the amount of brood maintained on the scanner surface and the factors contributing to its decline. Initially, a large number of cells became available simultaneously, indicating the queen's visits to the comb and resulting in a significant peak in egg-laying (Fig. 3, top). Subsequently, only individually vacated cells were filled, leading to smaller peaks. As the season progressed, the queen reduced her laying activity and new brood was rarely observed.

Approximately 24 days after major egg-laying events, significant hatching events followed (Fig. 3, middle), which later became more scattered. Fig. 3 (bottom) shows both, strategic brood removals, with over 30 drones removed per day, and individual removals targeting specific cells.

When synchronizing brood development, as shown in Fig. 4 (bottom), two periods of high brood removal become evident: one before and one after cell capping at day ten. Most of the brood was removed between day 5 and 7 (39 %) and between day 10 and 12 (35 %).

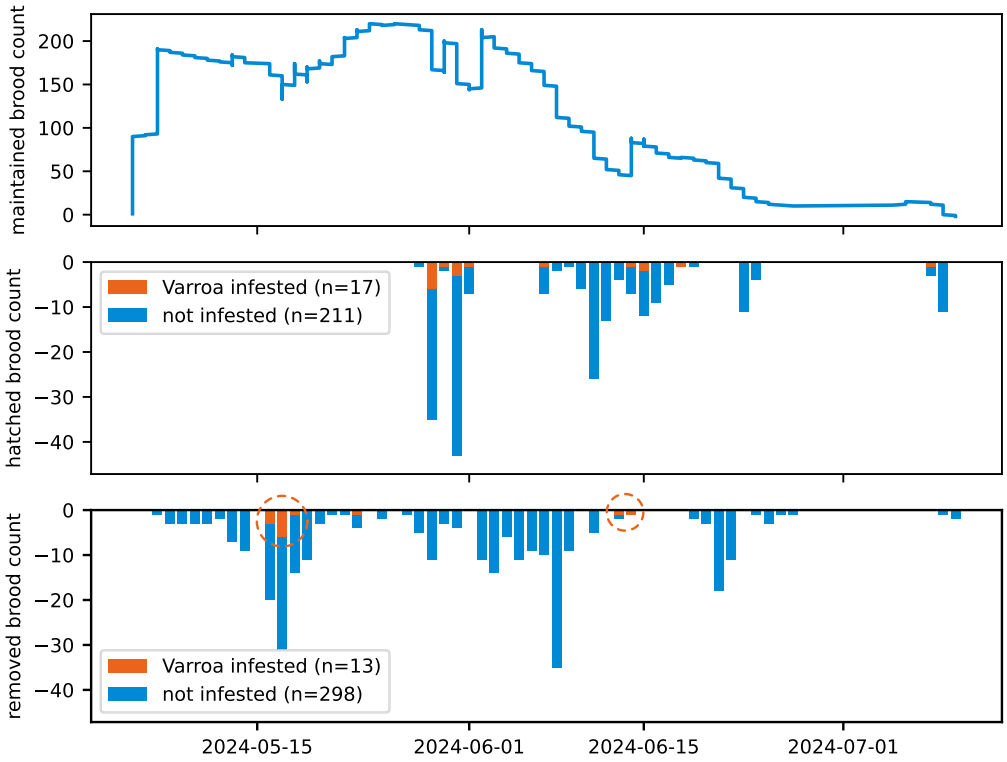

Fig. 3. (Top) The number of (Varroa infested) brood that was maintained at the given point in time. The number increases when new eggs were detected and decreases due to hatched or removed brood. (Middle) The daily number of (Varroa infested) hatched brood. (Bottom) The daily number of (Varroa infested) brood removals. The dashed circles highlight the mass removal of drone brood with Varroa infestations ranging from 7-19 % (left circle) and the specific removal of Varroa-infested brood, with infestation rates ranging from 50-100 % (right circle).

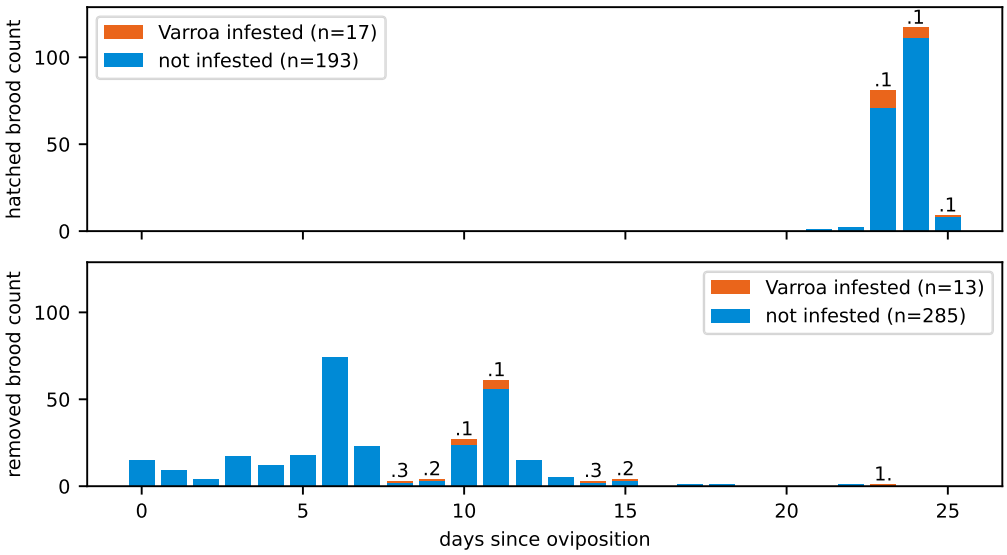

Fig. 4. (Top) The number of Varroa-infested drones that hatched at a given age. (Bottom) The number of Varroa-infested brood that was removed at a given age. Text annotations indicate the (rounded) fraction of infested brood, cell capping is expected at day ten.

3.4. Pathogen and parasite observation

Two larvae were infected by chalkbrood and 30 cells were invaded by Varroa mites. Fig. 5(a) shows a cell bottom covered with the white mycelium of the fungus *A. apis*, which causes chalkbrood. The first signs of mycelium appeared on June 2, 2024 at midnight, roughly five hours before the image in Fig. 5(a) was captured. Thirty minutes later, the bees began removing the infected brood.

Varroa mites were regularly first spotted swimming (and being trapped) in larval food. Fig. 5(b,c) showcase such an incident, which is further discussed in Ifantidis [20], lasting until the larvae consumed enough food to release the parasite. Since mites can move within the cells, they (including their offspring) are not always present at the cell bottom. Fig. 5(d) shows the foundress mite (red circle) and her offspring (white circle) passing by the image sensor.

Fig. 6 shows the visibility patterns of adult Varroa mites and their offspring across all 30 infested brood cycles. Most adult mites were initially observed before cell capping, often trapped in larval food (around day 10). Offspring were consistently first detected by day 16 at the latest.

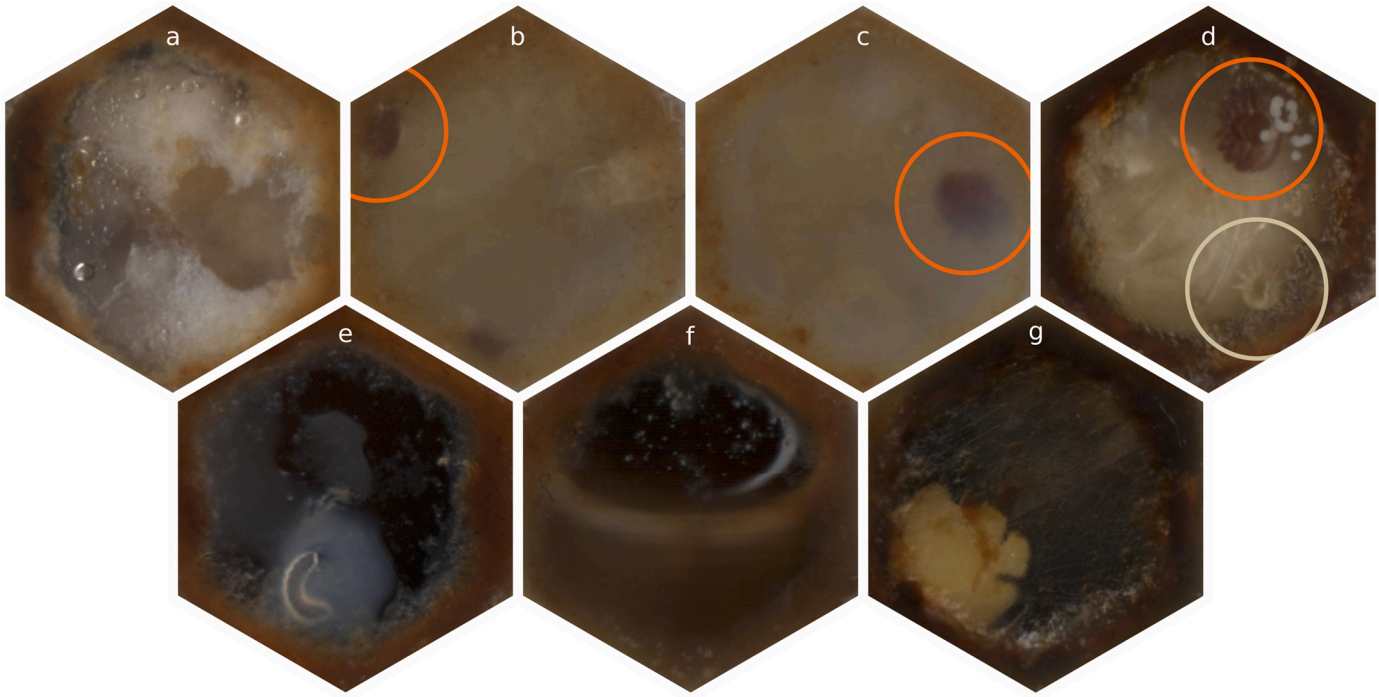

Fig. 5. (a) Chalk brood fungus evolves in a drone brood cell. Image was taken hours before the brood was removed and the cell was cleaned. (b, c) Adult mites (red circle) trapped in larval food. For an in-depth explanation of this pattern refer to [20]. (d) Foundress with offspring (white circle). White dots indicate the defecation of the mother mite, which is typically piled. (e) A recently hatched larva. (f) Nectar and (g) pollen stored in drone cells. Full resolution images can be found in the supplementary material.

In contrast to the trapped mites in Fig. 5(b,c), Fig. 5(d) show adult and hatched mites that can move freely. Note the white dots beneath the mother mite, which are excrements.

3.5. Beebread and stored pollen

Throughout the entire trial, empty cells were used to store nectar, a well-known and desired practice to ensure that the food supply is kept close to the brood nest. However, it was surprising that nectar was rarely stored for more than a few hours before being emptied or refilled. Towards the end of the drone season, drone cells became increasingly superfluous and were repurposed for minor storage of beebread. Fig. 5(f,g) illustrates the storage of nectar and beebread.

4. Discussion

Current methodologies for brood cell observations in honey bee research are destructive and unsuitable for continuous monitoring, which disturbs the study subjects and limits the practicality of long-term research [5,6]. This study aimed to address these limitations by developing a non-destructive, continuous monitoring technique using a modified flatbed scanner. Filling this gap is crucial for advancing our understanding of honey bee health, particularly in studying larvae, pupae, and Varroa mite infestations.

A key result of this study was the successful detection and monitoring of Varroa mites and *A. apis* in vivo, which enabled the achievement of unprecedented sample intervals of minutes without additional costs. These observations align with literature indicating that mites often hide at the cell bottom and exhibit movement patterns related to their developmental stages and the host larva [21].

To assess whether Varroa mites remain hidden from the sensor for extended periods, it is essential to analyze their visibility patterns. We observed frequent Varroa detections in most infested cells, enabling a robust analysis, particularly in cells where foundresses successfully reproduced. Rare detections of Varroa mites were mainly observed in cells that were cleaned out early, leaving little opportunity for detection.

The first observation of offspring frequently coincided with the host larva's molt on days 14/15, which may have prevented earlier visits to the cell bottom. Continuous visibility of the offspring aligned with their immobile developmental stages, during which some mites remained at the cell bottom (see Fig. 6). Extended periods of adult mite visibility were associated with atypical behavior. For example, the foundress in cell 60 fell into a state of agony and ceased activity; the mite in cell 100 died in the larval food, never being freed or removed; and the mite in cell 102 entered unusually early, remaining in the food for an extended period before being removed along with the brood. The mite in cell 269 was alive but inactive for no apparent reason.

We conclude from the limited data we have available, that it is unlikely for our method to miss infested cells with successful mite reproduction. While the likelihood of missing infestations increases when the observation period is shortened due to early brood removal, we estimate this risk to be minimal, as adult mites are often trapped in the larval food early on, making them clearly visible to the scanner.

Our method allowed for the simultaneous observation of up to 2500 brood cells, thereby significantly increasing the sample size in comparison to traditional methods [7,22,23]. The high-resolution images captured were of exceptional clarity, displaying even the smallest mite offspring and early-stage fungal infections.

This continuous monitoring approach is in accordance with previous research that has advocated for innovative techniques to improve infestation measurements and pathogen detection accuracy [24]. The advancement is particularly significant in light of the challenges highlighted by Lefebre et al. [8], where achieving sufficient single infested cells for reliable mite-non reproduction (MNR), reduced mite reproduction (RMR), and decreased mite reproduction (DMR) calculations proved difficult. This underscores the need for more efficient monitoring methods. Furthermore, our method corroborates the findings of McGruddy et al. [25], who demonstrated the efficacy of RNA interference (RNAi) in reducing mite reproductive success. Our approach provides a complementary method for observing and validating these biological effects.

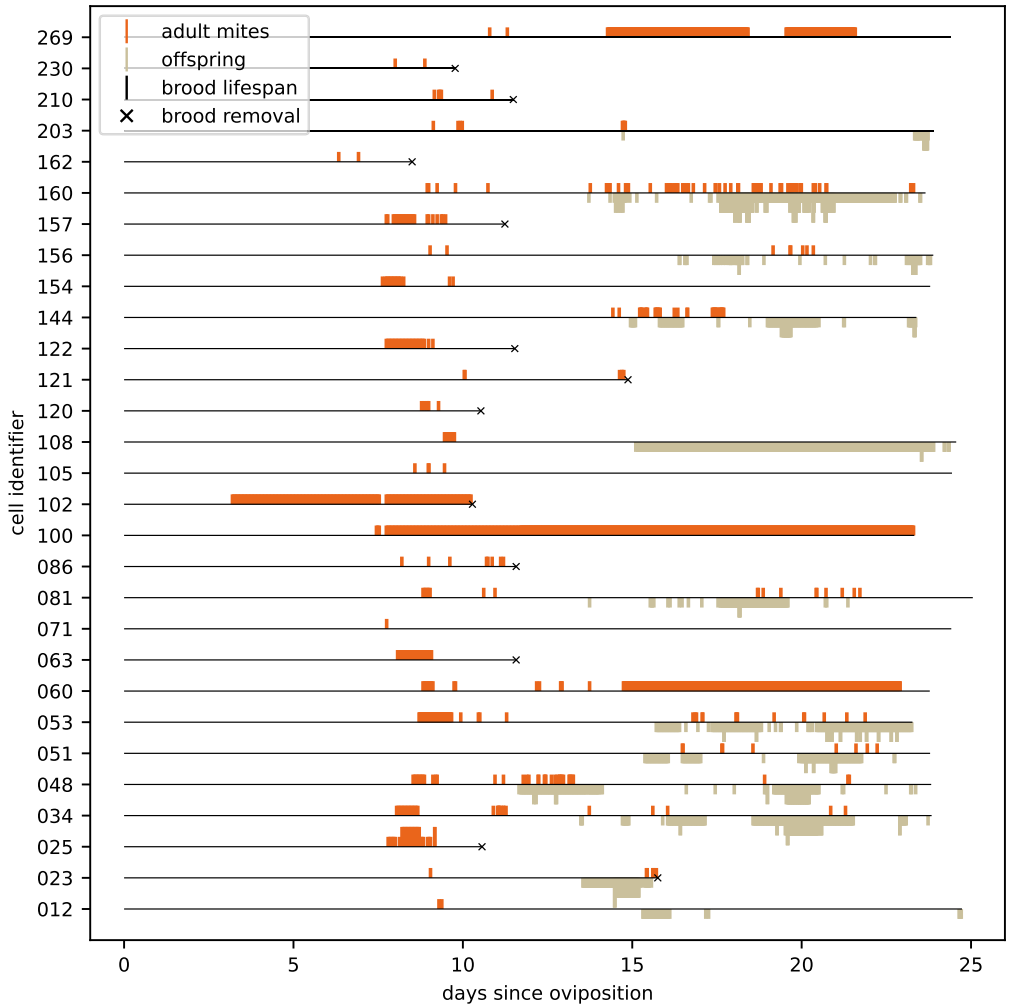

Fig. 6. For all infested cells (y-axis), black lines depict the brood's lifespan, while grey and red bars indicate the periods when parasites were visible to the annotator. Multiple detections at the same time are stacked; for instance, in cell 160, up to three offspring were observed simultaneously, and in cell 025, two adult mites were visible at the same time. Brood removals are marked with ×-symbols.

Furthermore, Siefert et al. [26] applied video recording techniques to examine honey bee brood care behavior in the context of chronic neonicotinoid exposure, thereby identifying notable discrepancies in nursing behavior and larval development. This scanner-based approach refines prior techniques by offering non-destructive, high-frequency monitoring, thereby expanding the scope of our ability to study these critical behaviors and their environmental stressors in greater detail in full sized colonies.

The findings of our study offer valuable insights into the natural behaviors of honey bees and their interactions with pests and pathogens. For example, the humid season facilitated the development of *A. apis*, which resulted in the formation of chalkbrood. Our continuous monitoring system was able to detect this early, providing an advantage over traditional weekly inspections. This finding is consistent with the literature, which indicates that less severe chalkbrood infections are often overlooked by beekeepers due to the rapid removal of mummified brood by worker bees [27].

Our scanner-based technique offers advantages over previous methods, such as macro video recording setups that required special lighting and limited the number of observable cells [26]. Unlike visual inspection techniques that focus on mites visible on the exterior of bees [28–31], our method detects mites within the brood cells. Therefore, it is not affected by mites hidden beneath the bee's sclerites [32], thus providing a more comprehensive assessment. [33].

Moreover, the device's capacity to monitor brood cell removal events revealed a notable occurrence of mass brood removal, which was likely attributable to external factors such as disease, food scarcity, or space constraints affecting all drone brood in a similar manner. This observation is of great importance for breeding programs that aim to promote VSH traits, as it allows for the differentiation between mass removal events and selective hygienic behavior directed towards infested cells [34]. For example, a brood removal on June 14, 2024 stands out as it was the only removal on that day, targeting a single Varroa-infested cell among an average of 69 brood cells. This makes it very unlikely to have occurred by chance (1.44 %; see Fig. 3 bottom).

Within a cell, two spots are crucial for the mite's reproduction: the feeding spot where the foundress bites the larvae and her offspring nourish themselves, and the defecation spot where the male and female offspring mate. The bite can be made visible using chemicals but cannot be seen with the naked eye, making it undetectable in the acquired image data. The mother mite defecates at the same spot, forming a pile of white excrement. Infestation can be quickly verified by searching for such excrement on the walls when infested cells are opened. Unexpectedly, one foundress preferred the floor over the cell's walls for defecation (see Fig. 5). Since all members of the mite family visit this spot frequently, this observation is particularly interesting, and the corresponding timelapse video is included in the supplementary material.

Our data showed that worker bees selectively removed Varroa-infested cells, thereby corroborating previous findings on VSH behavior.

This result is consistent with the observations of Sprau et al. [9], who demonstrated the complexity of the cues utilized by honey bees to detect and remove mites from brood cells, including movement and odor. Their findings highlight the importance of comprehensive and ongoing observation to elucidate the subtle mechanisms underlying VSH behaviors, supporting the value of our non-invasive imaging methodology. Furthermore, these insights align with the recent work of Morin and Giovenazzo [35], who identified a range of traits, including mite-non reproduction, recapping activity, and hygienic behavior, as key predictors of mite infestation levels. The intricate and dynamic nature of these behaviors underscores the need for non-destructive, comprehensive, and continuous monitoring approaches to accurately assess and enhance these traits in breeding programs.

5. Limitations

Despite its promising findings, this study has limitations. The sample size and scope were constrained by the available resources, which may affect the generalizability of the results. We observed that bees took approximately three weeks to draw out cells and lay eggs, a delay influenced by factors such as weather conditions and materials used.

The narrow depth of field and the temporal resolution of the scanner, although suitable for our purposes, might limit its applicability in other research contexts. Moreover, the substantial data size generated by high-resolution images necessitates efficient data management strategies, which could be challenging in larger-scale implementations.

Visual inspection at the opened hive during scanning showed no signs of disturbance whatsoever. Also, no signs of agitation were heard outside the hive at night when the sensor was triggered and the apiary was in complete silence. This suggests that the light and sound emitted by the scanning process did not cause significant disruptions. However, it should be noted that operating an electronic device inside a hive remains inherently invasive. As mites react strong to artificial rearing in the laboratory [5,6] similar effects must be considered and analyzed before employing the device in the field for scientific data collection.

Cleaning of the glass during the experiment was neither possible nor required. However, we did observe the accumulation of dirt, especially in unused cells. Cells that are regularly used for brood or nectar storage are effectively cleaned by worker bees and by the larva's food consumption, both enhancing the transparency. Almost no cell degraded in a way that made observation impossible over a period of three months. In the supplementary material, we included a time-lapse video of the cell that contained the highest number of eggs, illustrating the accumulation of debris and the bees' cleaning behavior.

6. Future work and applications

Future studies should focus on scaling this monitoring technique to larger bee populations and testing its effectiveness across diverse environmental conditions to ensure its adaptability to various beekeeping scenarios. A critical research area involves assessing the long-term impacts of continuous scanning on colony health and behavior, examining elements such as scanner light, electronics, and operational noise. Additionally, foundation design could be optimized by integrating wax scaffolding directly onto glass surfaces to improve bee acceptance of the foreign material.

While initial results suggest promising mite detection accuracy, comprehensive validation studies are still essential. As successfully done elsewhere in apidology [36–39], we plan to use the massive amount of collected and annotated data to automate the image data analysis utilizing artificial neural networks. Developing a suitable computer vision model would reduce processing time and make brood cell scanning more practical for widespread application.

Potential applications range from supporting breeding programs focused on selecting Varroa Sensitive Hygiene (VSH) traits to improve Varroa management, to enabling large-scale studies that could incorporate complete brood comb replacements, offering new avenues for advancing research into honey bee resilience.

Further investigation into brood development factors—such as pesticide impacts, the influence of Varroa infestation on brood hatching, and conditions prompting mass brood removal—will enhance our understanding of colony health. Additionally, investigating disease and pest control mechanisms could shed light on colony responses to Varroa invasion and reproduction.

7. Conclusion

In conclusion, this study presents a novel, non-destructive method for continuous monitoring of honey bee brood cells, addressing a significant gap in current research methodologies. By enabling detailed, continuous observations of Varroa mites and *A. apis*, this scanner-based technique could significantly advance the study of honey bee health and pest management. The major contribution of this study lies in its ability to enhance the accuracy and practicality of field research, paving the way for improved beekeeping practices and more resilient honey bee populations. This work supports the overarching goal of mitigating the impacts of pests and pathogens on honey bee colonies, ultimately supporting the sustainability of apiculture and agricultural ecosystems.

CRediT authorship contribution statement

Parzival Borlinghaus: Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jörg Marvin Gülzow:** Writing – review & editing, Validation, Software, Resources, Methodology, Data curation, Conceptualization. **Richard Odemer:** Writing – review & editing, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT and DeepL to improve the wording and text structure of the manuscript for enhanced readability. After using these tools, the authors thoroughly reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.atech.2024.100655.

Data availability

All recorded cell events can be found in the supplementary material. Due to the large size, image data will be provided on request.

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