# Insights into Entomopathogenic Nematode Behavior by Using AI Techniques to Advance Sustainable Pest Control

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**Abstract**

Entomopathogenic nematodes (EPNs) are organisms that are often mass-produced as biological control agents (BCAs) to mitigate pesticide-related hazards and foster environmental sustainability. Enhancing our understanding of EPNs biology and their interactions with hosts is crucial for refining the use of EPNs in integrated pest management. This study pioneers an interdisciplinary approach, integrating engineering, and entomology, to investigate the behavior of *Steinernema carpocapsae* EPN. Employing a novel blend of deep learning and optical flow analysis, a Convolutional Neural Network (CNN) effectively recognizes how nematodes react to host-borne stimuli. Achieving remarkable precision (1) and an overall accuracy of 0.938, the model elucidates EPN behaviors in a prototyped microfluidic platform reproducing a host-environment context. The integration of optical flow analysis highlights an increased motor activity in EPNs when exposed to stimuli, adding novel information on their dynamic responses. This versatile methodology represents a significant advancement in detecting and understanding EPNs responses to diverse stimuli, fostering their use as advanced BCAs in sustainable pest control and environmental management.

**Keywords**

deep learning, optical flow, biological control, chemo-ecology, lab-on-a-chip

## 1. Introduction

In biological and ecological research, machine learning and lab-on-a-chip technology are offering a unique combination, providing deep insights into biological system processes [1]. In this multidisciplinary context, our study focuses on the understanding of the behavioral and motor displays of *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae), an entomopathogenic nematode (EPN) [2] known for its use as biological control agent (BCA) [3]. This species is described in the literature as a nematode exhibiting ambusher behavior [4]. However, our objective is to evaluate its ability to move and thus behave as a cruiser nematode when exposed to organic molecules. This new understanding could be beneficial both for enhancing ethological knowledge of this species and for expanding the range of potential hosts against which it could be employed as a BCA [5]. The approach proposed in our study aims at advancing understanding on biological control strategies and their applications in integrated pest management programs, aligning with the One Health and EcoHealth perspectives [6].

Microfluidic platforms offer a controlled environment for studying the organism-environment interactions, providing researchers to mimic and analyze complex biological processes [7]. In the field of behavioral research, many studies utilize methods that integrate microfluidics and computer vision, especially when studying model species or those with extensive locomotion capabilities [8]. In addition to these established approaches, we have introduced the use of Artificial Intelligence (AI) and deep learning to further explore behavioral differences in this context. AI-based techniques have demonstrated promise in examining motor anomalies in model organisms [9, 10]. Similarly, AI methodologies have enabled the utilization of model organisms as biosensors [11, 12].

Our comprehensive study employs a combination of established microscopic techniques with microfluidics, deep learning, and optical flow analysis, to investigate *S. carpocapsae* behavior and develop an automated workflow. First, infective juvenile stage specimens of *S. carpocapsae* were observed using an inverted microscope to evaluate their movement. Experiments were carried out in a microfluidic environment where they were exposed to organic compounds obtained from the feces of *Lobesia botrana.* An arena, designed using fast prototyping techniques, aimed for optimal nematode locomotion and

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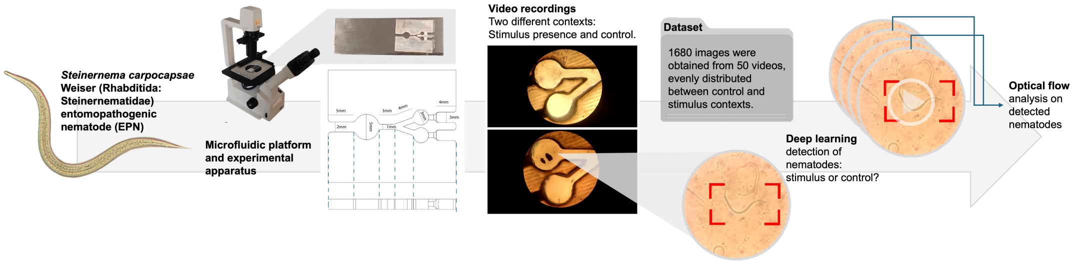


**Figure 1:** Workflow of the proposed approach.

minimal turbulent motion. A deep learning approach differentiated between scenarios involving stimulus presence and control conditions, enabling the analysis of motor differences attributed to the stimulus presence. Nematodes are utilized as biosensors to gather environmental information and investigate changes in behavioral traits in response to stimuli. The integration of optical flow analysis revealed distinct variations in motor activity among individuals across different contexts. This study bridges microfluidics, machine learning, and sustainable integrated pest management, offering insights into *S. carpocapsae* EPN behavior. It contributes to the broader context of the convergence of AI, engineering, and entomology, advancing strategies for biomedical research and sustainable pest control.

## 2. Methods

### 2.1. *Steinernema carpocapsae*

We used NEMOPAK SC, a commercial product distributed by BIOPLANET (Cesena, Italy), to obtain and test *Steinernema carpocapsae* nematodes at infective juvenile stage (IJ). Nematodes were maintained at 4-8°C till the expiration date and solubilized in water at 25°C, with constant stirring and heating, obtained using a magnetic vortex, to keep them viable before trials.

### 2.2. Tested cue

EPNs are attracted by chemical cues deriving from potential hosts. Some studies show the attractive effects of gut content of hosts on EPN locomotion [13]. Thus, we used *Lobesia botrana* 5th instar larvae feces to investigate responses in *S. carpocapsae*.

### 2.3. Microfluidic platform and experimental apparatus

The miniaturized testing arena was designed in SolidWorks (Dassault Systemes, Velizy Villacoublay, France) and then fabricated by rapid prototyping in a biocompatible resin (VisiJet® M3 Crystal, 3D Systems). The microfluidic testing arena, featuring a releasing chamber (Ø=5mm; *h*=2mm) and two stimuli chambers (Ø=3mm; *h*=2mm), creates small environments for the analysis of nematode behavior without imposing spatial constraints on their motility, thereby avoiding potential biases. Each stimuli chamber is connected to the releasing chamber through an aisle creating an y-maze arena. The floor of the test arena is represented by a transparent glass plate firmly connected to the base of the upper component by depositing and curing a polydimethylsiloxane (PDMS) film (Sylgard 184). To observe and record the behavior of nematodes, an operator introduced the nematodes into the releasing chamber using a pipette. Subsequently, the miniaturized testing arena was carefully positioned under a 3D visual inspection microscope (Nikon TMS) with a total magnification of 25X. This setup allowed for precise and detailed observation of EPN behavior within the microfluidic system, ensuring accurate recording of their responses to the specific stimuli provided in the chambers.

### 2.4. Experiment workflow and recordings

The nematodes were reactivated in water at a temperature of 25°C for 20 minutes as arranged by Bioplanet srl. (Cesena, Italy). Meanwhile, the microarena was washed with water and 70% ethanol to remove contaminants, reduce surface tension, enhance arena wettability, and improve nematode locomotion. A second washing was carried out using water to eliminate the alcohol traces potentially harmful to the EPN. The chip was then filled with the medium, 0.9% NaCl physiological solution within which the nematodes move, it is useful to avoid osmotic stress. Solution and nematodes were inserted using a plastic micro pipette. The chip thus assembled is placed under the microscope and the stimulus is positioned in the opposite arenas, alternating localization to avoid bias during the analysis. The videos were recorded under the microscope for a total of 5 minutes. Video analysis considers only 60 seconds recording to avoid acclimation time and promote next computer vision and deep learning analysis. Videos are recorded by using an RGB camera (48MP, $f/1.8$) set on the microscope. Following each test, the device is washed and cleaned in accordance with the previously described procedures.

## 2.5. Deep learning detection

A deep learning approach was utilized to differentiate between scenarios involving stimulus presence and control conditions for nematodes. Beyond discriminating between the two contexts, this approach enabled the analysis of motor activity differences captured through images, attributed to the stimulus presence. In this sense, nematodes have been employed as biosensors to gather information about the surrounding environment, while also investigating changes in behavioral traits in response to a stimulus using deep learning techniques. To achieve this objective, a YOLOv8n Convolutional Neural Network (CNN) model pre-trained on the COCO dataset, a widely used benchmark for object detection tasks [14], was utilized. The selection of this CNN model was driven by its versatility and demonstrated effectiveness in object detection tasks. YOLO networks have been applied across various detection tasks, particularly in the v8 version, encompassing small object identification in Unmanned Aerial Vehicle (UAV) images [15], evaluating medical face mask adherence in COVID-19 scenarios [16], or also detecting diverse marine species [17]. YOLOv8 employs a CNN structured into three main components: the backbone, neck, and head. The backbone, based on a modified CSP-Darknet53 architecture, facilitates multi-scaled object detection through a feature pyramid network, generating five scale features [18]. The neck introduces a PAN-FPN architecture, optimizing performance while maintaining a lightweight design. The detection head consists of convolutional and fully connected layers for object classification and bounding box regression. Loss functions like BCE Loss and DFL/CIoU are employed. YOLOv8 operates as an anchor-free detection model, directly predicting object centers. Dynamic sample assignment is achieved through the Task-Aligned Assigner mechanism, further improving accuracy and robustness. Architectural enhancements include module exchanges and convolution replacements, resulting in a model weighing 6.24 MB in the v8 nano version. The pre-trained CNN model was fine-tuned to identify and distinguish nematodes in the presence of a stimulus from those in a control setting. A dataset of 1680 images was obtained from 50 videos, evenly distributed between control and stimulus contexts. The dataset was divided into training (70%), validation (20%), and testing (10%) sets, resulting in subsets comprising 1189, 339, and 152 images, respectively. An equal distribution of images across both classes was ensured within each subset. Manual labeling of images was performed using the online Makesense software. The dataset also includes approximately 10% background images without nematodes, distributed in the same 70-20-10 ratio. The CNN was trained with a batch size of 8, over 200 epochs, and an image size of 1920x1080 to enhance data quality when dealing with small objects. Afterward, 32 one-minute inference videos were analyzed, evenly split between stimulus and control contexts. Frames were extracted from each video at a frequency of 1Hz. For each frame, the CNN detected nematodes distinguished under stimulus and control conditions. The outcomes were averaged for each frame to establish the presence or absence of a stimulus in the scenario. This procedure was iterated for each video. In evaluating the CNN model, various metrics such as accuracy, precision, recall, f1-score, and mAP were considered. Training and subsequent analyses were performed using Ultralytics in Python with a Tesla T4 GPU.

## 2.6. Optical flow analysis

For a comprehensive motor activity investigation, an optical flow analysis was integrated into the deep learning framework, using the Farnebäck algorithm [19] with specific parameter settings (image scale of 0.5, 5 pyramid layers, averaging window size of 15, 3 iterations, size of the pixel neighborhood used for polynomial expansion in each pixel of 7, standard deviation of the Gaussian used to smooth derivatives for the polynomial expansion of 1.5). A total of 60 videos, balanced between control and stimulus contexts, were considered. Given that nematodes exhibit body movements with a 2Hz frequency according to literature [20], frames were sampled at 5Hz in accordance with the Shannon theorem for the optical flow analysis. First, the optical flow magnitude was computed by comparing two frames. The CNN model was exploited to obtain the bounding boxes of the detected nematodes. Then, the mean magnitude value for each bounding box was considered. Lastly, the maximum magnitude among the averaged values of the bounding boxes was extracted for each frame, to avoid misclassifications with background. Temporal data were collected, and the mean of these values was computed to derive a representative value for each video. The analysis was conducted using OpenCV in Python.

## 2.7. Statistical analysis

Data were normally distributed (Shapiro-Wilk test, $p>0.01$) and homoscedastic (Levene test, $p>0.01$). Therefore, statistical significance between stimuli and control was established using a $t$-test. Statistical analyses were performed using JMP Pro 17 software. The threshold was set at $p=0.05$.

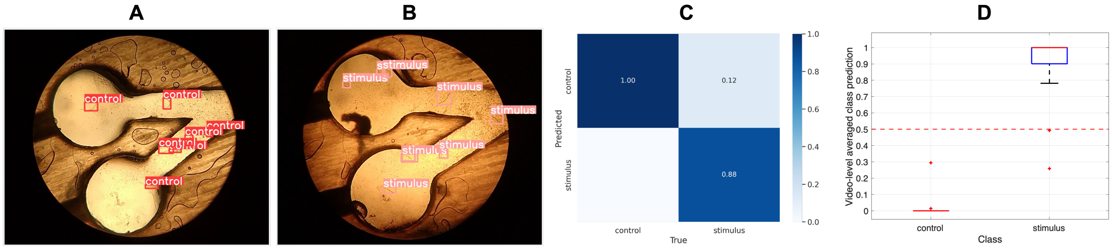


**Figure 2:** Deep learning detection results: frame-level predictions for control (**A**) and stimulus (**B**) scenarios; along with the video-level normalized confusion matrix (**C**) and averaged class prediction values (**D**).

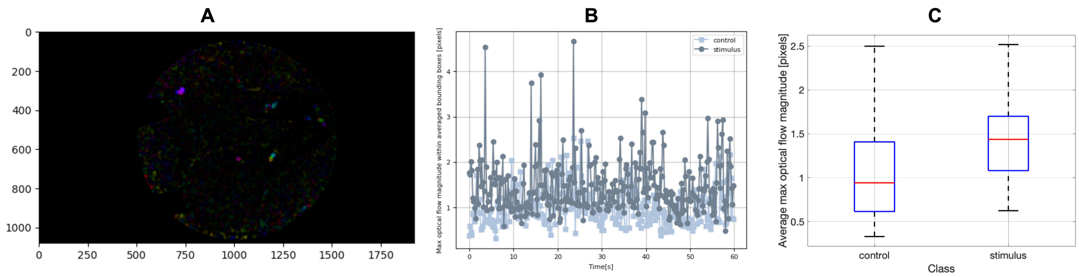


**Figure 3:** Optical flow analysis results: graphical representation of magnitude for a stimulus context sample (**A**), based on two frames sampled at 5Hz; for two video samples, comparison of temporal profiles (control and stimulus contexts) of optical flow magnitude, evaluating the maximum value within bounding boxes averaged values for each frame (**B**); comprehensive video-level analysis considering the mean values of the temporal profiles obtained within the video dataset (**C**).

# 3. Results

Microscopical observations have allowed us to identify the main behaviors performed by *S. carpocapsae*, including an increase in movement and the size of oscillations. This clearly demonstrates the presence of an effect due to tested cue arising from the host on the gestural complex of the EPN. Our findings indicate that *S. carpocapsae* exhibits not only a sit-and-wait strategy but also demonstrates active host-seeking behavior in response to chemical stimuli within its proximity. The fact that nematodes changed their posture and locomotion direction towards the stimulus is functional to describe *L. botrana* feces as an attractive cue. Furthermore, this characteristic has been sparsely evaluated in the literature [21] but holds significant value for enhancing our understanding about this species ethology and optimizing its utilization as BCA. The CNN model achieved a precision of 0.632, recall of 0.623, and mAP0.5 of 0.608 on the validation set. Predictions for two images outside the training and validation processes are shown in Fig.2(**A**) and (**B**). Although the network exhibits limitations, particularly in individual identification compared to background, conducting video analysis yields promising results with no errors in identifying the control context, as demonstrated in the normalized confusion matrix in Fig.2(**C**). Examination of the normalized confusion matrix indicates that the control class achieved 100% accuracy, while the stimulus class exhibited 88% accuracy with a 12% error attributed to misclassification as the control class. Fig. 2(**D**) showcases predictions in video analysis. Overall, the video analysis attained an accuracy of 0.938, precision of 1, recall of 0.875, and f1-score of 0.933 in identifying stimulus presence from behavioral traits. The results obtained through deep learning validate the observations. The CNN effectively distinguished between the two contexts (stimulus presence and control) based on nematodes. Considering this image-based approach, the differences are attributed to altered gestures due to the presence of a stimulus. The subsequent analysis integrating the optical flow aims to investigate changes in motor activity over time between the control and stimulus contexts. Fig.3(**A**) shows a graphical representation of the magnitude for the stimulus scenario, obtained through MINMAX normalization. Initially, the CNN model was utilized to obtain bounding boxes for each frame. Subsequently, the mean optical flow magnitude was calculated for each bounding box. Finally, the maximum value among the averaged magnitude values in the bounding boxes was determined. Temporal profiles of this value for the control and stimulus contexts are compared in Fig.3(**B**) considering two video samples. The mean of these magnitude values over time was considered to obtain an overall value for each video. Fig. 3(**C**) illustrates the comparison between control and stimulus data. Statistical analysis conducted on this data demonstrates significance ($p$=0.010), providing evidence of observed differences in motor activity. The integration of optical flow with deep learning detection reveals increased motor activity of nematodes in the presence of the stimulus when compared to the motility of control specimens.

## 4. Discussion

The deep learning approach effectively differentiated between scenarios with stimuli and control conditions for nematodes. Beyond discrimination, it helped analyze motor activity the presence of the stimulus. EPNs have been used as biosensors, gathering environmental data and revealing behavioral changes through deep learning techniques. We utilized various methods, including microfluidics, deep learning, and optical flow, to investigate these differences and develop an automated workflow for studying EPNs. Microfluidics was already employed in several biological and behavioral assays with different species [22], but considering nematodes, this technique is common also for the model organism *Caenorhabditis elegans* [23]. Our innovative approach is essential for studying a parasite's behavior and locomotion in a natural-like condition. AI analysis revealed challenges at the frame level due to background interference, but exploring different architectures may improve results. However, at the video level, the model showed satisfactory performance with 100% precision. Optical flow and statistical analyses highlighted distinctions in motor activity between individuals in different contexts. Tests on EPN movement emphasized their ability to sense, move towards, and enter hosts. AI-based results confirm the preliminary microscopic observations regarding the presence of active motility in the tested species and supports the few assays which consider *S. carpocapsae* a nematode with some cruiser's features [21]. Considering our results it's clearly observed that *S. carpocapsae* has an actual locomotion towards the stimulus, explainable as chemotaxis where there is a spatial movement and klinotaxis where speciemens change their body conformation by bending or turning in presence of the organic stimulus. Nematodes demonstrate aggregate migration; notably, we observed increased presence near the attractor [23]. According to our results, we are able to define larval feces cues as a kairomone; in fact, they evoke a physiological response in EPNs, which is favorable to the receiver but not to the emitter. Nematode responses were assessed using automated motility tracking and computer vision techniques [24]. The proposed integrated methodology significantly enhances our ability to discern and understand EPN responses to diverse host stimuli. The use of AI-based techniques and deep learning, integrated with microfluidics and optical flow analysis, represents an innovative approach to behavioral studies, offering the possibility of using nematodes as biosensors. Further research on EPN behavior, utilizing pose estimation and diverse organic stimuli, could enhance commercial product performance and expand *S. carpocapsae's* use as a BCA for other phytophagous species, supporting biological control principles [5].

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