CONSIDERATION ON HOW TO IMPROVE GROUND REACTION FORCE MEASUREMENTS IN SMALL WALKING INSECTS

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Micro-vibrations caused by the motion of insects, provide a content-rich signal that may be perceived by nestmates, competitors or predators. Knowing the ground reaction forces of a single leg impacting the surface can provide quantitative information about the interaction with the substrate, the substrate itself, physiological and behavioural state of an individual, through mechanistic constraints and the diversity of the gait. Micro-force plates have been used for measuring the ground reaction forces in the order of micro-Newton, using highly sensitive strain gauges attached to compliant load-bearing parts of an underlying mechanical structure. However, their calibration and signal-to-noise-ratio are some of the main challenges of designing these highly sensitive systems. For fine movement analysis, the micro-force plates need to be coupled to high speed video recording systems; the synchronisation of the camera and force plate represents another challenge. For an existing micro-force plate designed for ant measurements, which showed linear signal response in the calibrated force with a lower limit of 120 µN, the linearity of force measurement and sensitivity of the device are investigated in a lower force range, extending the opportunity to study also insects with a lighter footfall. We take into account the difficulties of adapting such devices to the insects' needs related to the environment (i.e. temperature, light...) and morphology (i.e. dimension, weight...). Based on the experiments of the force plate, we consider how to design an experimental setup that overcomes many of the behavioural and technical challenges, to enable more efficient and accurate measurements for insects with body weights less than 5 mg.

Keywords: force-plate, substrate, insect, behaviour, ground reaction force, biotremology

1. Introduction

Most animals are able to produce and perceive vibrational signals to exchange information. In fact, vibrational communication (biotremology) is considered one of the most ancient modalities of communication [1]. Among insects, a continuously increasing number of species are discovered to communicate through substrate-borne mechanical waves. Surprisingly, for some of them it emerged as their major, if not the only one, communication channel [2], [3], [4]. Vibrational signals may be produced specifically to manipulate receiver behaviour (i.e. feeding, mating, alarm), while other unintentionally produced vibrations can transmit cues that represent a passive, non evolving source of information [5], [6]. In this scenario, micro-vibrations caused by the motion of insects, provide a content-rich cue that may be perceived by nestmates, competitors or predators. For example, termites can eavesdrop on vibration produced by competitors to avoid confrontation [7]. Those micro-vibrations can be produced by several activities, such as chewing or walking, and provide information not only about the number of individuals, the activity level and location of other animals, but also about the behavioural and physiological state of the insect and about the mechanical properties of the substrate.

Therefore, collecting information about these variable and low amplitude vibrations, such as the ones generated by the walking activity [8], is arduous. Beyond the challenge of understanding and interpreting the communication signal itself, it has been the focus of research [9], while signal generation (actuation), measurement (sensing), and neural processing are less often studied. Whereas there are diverse methodologies that can be employed to record vibrations, such as accelerometers or laser vibrometers, it is difficult to separate the response of the substrate from the signal itself. A first attempt to isolate the excitation signals has been conducted through inverse methods via blind-deconvolution using a model of a substrate [10] and extracting ant signals. Further, due to difficulties in modelling the substrate properly, since timber is highly nonlinear, heterogeneous material, deconvolution with Tikhonov regularisation has been conducted directly by using the transfer function of a measured veneer, and filtering a recorded ant footstep response by applying geometric filtering using the dynamics phase space [8].

Measuring ground reaction forces is considered an indirect approach as the reaction force, according to Newton's laws, is assumed to be equal and opposite to the excitation force [11]. It is considered a forward approach as the excitation signal is measured without the need of deconvolution [12]. Micro-force plates have been employed in several studies to investigate locomotion in ants or cockroaches [13]–[15]. An example is the micro-force plate designed by Reinhardt et al. [16] to investigate forces induced by ants walking. The device is equipped with commercially available semiconductor strain gauges of type KSP-3-120-F2-11 (Kyowa, Tokyo, Japan) and connected to a digital multi-channel amplifier system ML10B (Figure 1a). The micro-force plate showed linear signal response in the calibrated force with a lower limit of 120 μN for a single leg. The device is enclosed by an aluminium housing, that has on the surface, an arena designed to observe insect walking behaviour [16].

The same device has been tested to evaluate the linearity of force measurement and sensitivity in a lower force range (up to 10 μN), extending the opportunity to also study insects with a lighter footfall (Reinhardt and Blickhan 2014). The procedure to investigate the sensitivity of this device represents a challenge itself. It is susceptible to errors due to the difficulty of placing by hand the weight on a very small sensor without producing air movements and changes in the temperature.

We use a multidisciplinary approach to identify major difficulties, not only related with the device properties (engineering) but also to the insects' physiological state (biology). Furthermore, we propose improvements to overcome the identified difficulties in the calibration process, to optimise the force plate and the arena and to satisfy the insect's biological requirements.

2. Materials and methods

2.1 Static Calibration

To proceed with the device calibration a number of weights were manufactured using iron or aluminium materials (Table 1). Each weight was measured accurately to 0.1 mg using an analytical balance (ABS 80-4, Kern and Sohn, Balingen, Germany). After approximately 5s from the start of the recording (to reach minimal drift in voltage signals), each weight was gently placed on the platform using a tweezer. After another 5 seconds, the recording was stopped, and the weight was removed from the sensor. The same procedure was repeated up to six times for each weight and for measurements of force in all directions (*x, y, z*). In order to measure forces in different directions the device was rotated so the gravity acted in the direction to calibrate.

The signals were recorded by a data acquisition system with a built-in amplifier (MGCplus, Hottinger Baldwin Messtechnik, Darmstadt, Germany) at a sampling frequency of 1 kHz. The data acquisition hardware was connected through a USB communication processor (CP22) to a desktop PC running the data acquisition software Catman Easy V3.3.3 (Hottinger Baldwin Messtechnik, Darmstadt, Germany) (Figure 1a).

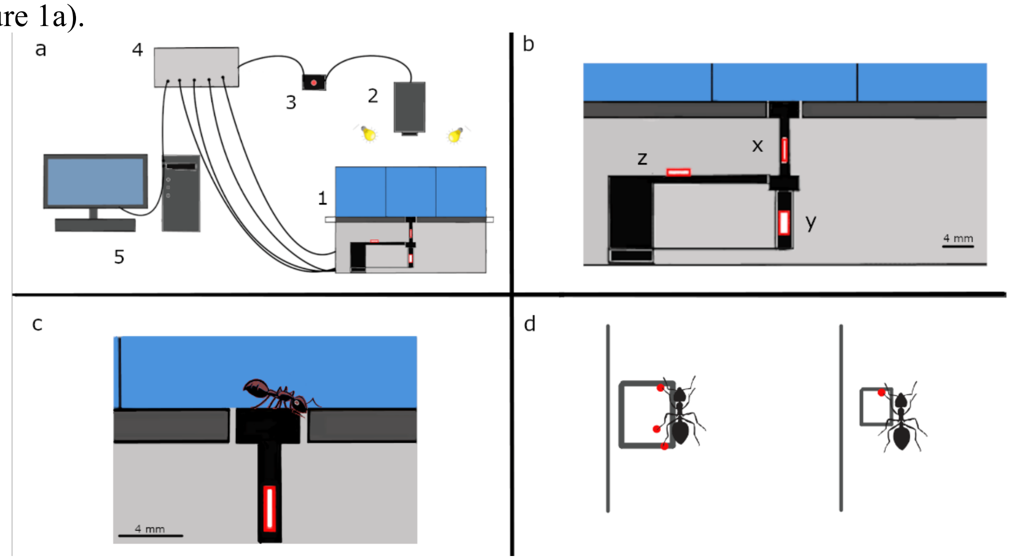

Figure 1: a) schematic of the set-up; 1) Arena with force plate, 2) High speed camera and lights, 3) Trigger, 4) Amplifier, 5) Computer; b) Schematic of the force plate beams and strain gauges position; c) The insect might enter inside the device through the gap between the force plate and the arena, d) Importance of scaling the stepping surface of the beam based on the insect dimension.

Table 1. Mass of the small weights used for static calibration. The force data corresponds to the mass data, that is measured using the analytical balance, multiplied by the gravitational acceleration of 9.81 m/s^2. The third column shows the direction of the applied force on the micro-force platform. *N*= number of repeats

Load Force (µN)	Mass (mg)	Direction (*x, y, z*)	*N*	Load Force (µN)	Mass (mg)	Direction (*x, y, z*)	*N*
987	100.6	0, -1, 0	6	193	19.7	0, 0, -1	6
		1, 0, 0	6	153	15.6	0, 0, -1	6
		-1, 0, 0	6	106	10.8	0, -1, 0	6
698	71.2	0, 0, -1	6			1, 0, 0	6

		0, 1, 0	5			-1, 0, 0	6
647	66.0	0, -1, 0	3	96	9.8	0, 1, 0	6
493	50.3	-1, 0, 0	4	95	9.7	0, 0, -1	6
492	50.2	0, 0, -1	6	50	5.1	0, 1, 0	6
454	46.3	0, -1, 0	6	49	5.0	1, 0, 0	6
		1, 0, 0	6	48	4.9	0, 0, -1	6
378	38.6	0, 1, 0	6	33	3.4	0, -1, 0	5
373	38.1	0, -1, 0	5			-1, 0, 0	6
341	34.8	0, 0, -1	3	26	2.7	0, 1, 0	6
204	20.8	0, -1, 0	6	25	2.6	0, 0, -1	5
		1, 0, 0	6	13	1.4	0, 0, -1	6
		-1, 0, 0	6	9	1.0	0, 1, 0	5
202	20.6	0, 1, 0	6				

2.2 Data processing

Raw data was saved in TSX Format, which is a native format for the data acquisition software, and information such as the voltage signals of each measurement in time were extracted and processed using MATLAB 9.8.0 R2020a (The MathWorks, Natick, MA, USA). From each time series, the difference in the mean voltages before and after placing the weight was obtained. A linear curve fitting in MATLAB was applied for drift correction first and then to evaluate the linearity of the measurements.

3. Results

After calibration and processing of the measured voltage signals, the sensitivity matrix **K** was obtained to establish the relation between force and voltage for this micro-force plate. The coefficient values are given in the following equation:

$$K = \begin{bmatrix} -62.170 & 10.906 & -7.411 \\ -3.535 & -58.972 & -24.340 \\ 13.794 & -1.708 & 277.391 \end{bmatrix} \text{X } 10^{-5}\ \mu\text{N/mV} \quad (1)$$

The measured coefficients of the K matrix show less than 15% coupling between sensors, except for the coefficient (row 2, column 3), representing the contribution to Fy from the measured strain in the z-direction, with 40% coupling. Nevertheless, the direct sensitivity coefficients (diagonal terms) are larger than the others, as expected from this specific design of the micro-force plate [16]; hence, it is a fairly accurate estimation to correlate the force components to their corresponding strain measurement in the same direction, as expressed in the following equation:

$$F_x(U_x) = -62.170\ 10^{-5}\ U_x, \text{ with } r^2 = 0.98, N = 58,$$

$$F_y(U_y) = -58.972\ 10^{-5}\ U_y, \text{ with } r^2 = 0.99, N = 78, \text{ and} \quad (2)$$

$$F_z(U_z) = 277.391\ 10^{-5}\ U_z, \text{ with } r^2 = 0.99, N = 49.$$

where $\mathbf{F} = (F_x, F_y, F_z)$ denotes the force vector in μN and U is the measured voltage in mV. Mean sensor outputs versus loading force are shown in Fig.2.

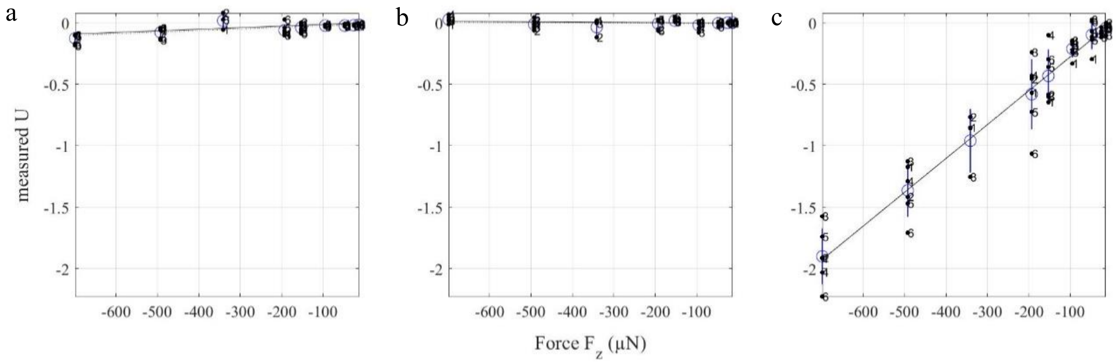

Figure 2: Calibration of the force plate in the *z*-directions using standard weights between 100.6 and 1.0 mg (987 - 9 μN); a) *zx*-direction; b) *zy*-direction; c) *zz*-direction.

4. Discussion

In this study we explored the linearity of measurements and sensitivity of an existing micro force plate in a lower force range. Compared to the 2014 results [16], it was found that the sensitivity matrix **K** shows more cross-talk between sensors, which can be due to the performance decrease of the load bearing mechanism, as a result of mechanical creep, and sensitivity of strain gauges over the past seven years. Nevertheless, we could measure voltage signal coming from the strain gauges when forces from 987 to 9 μN were applied. The linearity of measurements in aforesaid range is shown in Fig. 2, displaying that it is possible to obtain a fairly accurate measure of the force from the tests of insects walking over the micro-force plate. Based on the experience acquired during calibration, we propose some ideas to improve its protocol, in order to overcome some of the difficulties encountered during the procedure and improve the quality of the measurements.

One of the major challenges is represented by the difficulty of precisely placing the weight by hand on a very small sensor without overloading the micro-force plate, due to human contact while placing or removing weights during calibration, or without producing air movements and changes in the temperature. This procedure is time consuming and is susceptible to errors. To avoid this human error, we propose the adoption of micro-actuators. These devices are used to excite a structure on micro-force levels. Piezoelectric micro-actuators provide precise and fast linear motion, enabling accurate excitation of small structures. Travel range and force of these actuators can be controlled via input voltage levels, allowing the application of the desired force amplitude for an arbitrary amount of time on the force plate to monitor the recorded force results. Moreover, it is possible to excite specific parts of the force plate so that the calibration can be performed more efficiently and accurately. The actuator can also be used to play back recorded walking signals of an insect. This allows the comparison of measurements from the actual animal against the ones from the actuator, which can be used to validate the mechanical model of the insect walking and provide insights on their correct pedal gates. Another improvement could be evaluating the linearity of the force measurements during calibration for different ranges such as more than 100 μN, between 100μN to 10μN, and less than 10 μN, accounting for the response change of the system at different levels of force sensitivity.

In addition to calibration, we propose a number of adjustments to develop a device, which can allow force measurements and behavioural observations of insects with a lighter footfall, as summarised in Table 2. We take into account not only structural modification designed to increase the micro force plate sensitivity, but also the insect biology and behaviour to create the most suitable experimental environment. Despite being well acknowledged [17], the importance of conducting behavioural experiments under the most natural conditions possible is often underestimated. For instance, a stressed or alarmed animal might alter its walking pattern in response to the environmental conditions, as in the case of the cockroach *Periplaneta americana* that can switch from exapedal to quadrupedal and bipedal running if disturbed [13].

Therefore, a number of structural changes are suggested, such as increasing the beam length and decreasing its thickness. This provides a more compliant mechanism and thus increased sensitivity. Furthermore, both the provided stepping surface area at the end of the beam and the gap between the plate and the arena need to be decreased, to raise the chances that the insect steps on it with only one leg at a time, without the animal escaping inside the device (Fig. 1 c, d). The manufacturing of these adjustments can be a challenge, depending on the used materials.

Some of the considered improvement regard the type of strain gauges adopted. These can degrade depending on environmental conditions such as temperature and humidity, resulting in a progressive reduction of the accuracy with time. A possible alternative is to use glass optical fibres with polymer coating for strain measurement [18]. This can increase the lifetime of the micro-force plate and improve the accuracy of measurements as long as the changes in the environment temperature is less than a few degrees or the setup is placed in a controlled environment. The main challenge of using optical fibres is its attachment to the support of the load-bearing beams. This can be circumvented by using resin-based 3D-printing and integrating the sensor placement using an additional resin layer on the top of each fibre. The calibration and testing are the same as conventional resistive strain gauges.

To increase the quality of measurements and the efficiency of the device, not only the force-plate can be improved, but it is important to rescale the walking track dimensions according to the insect size. Thus, the insect can freely walk, but at the same time it does not spend too much time wandering around without stepping on the micro force plate.

Artificial lights are known to alter animals' behaviour in that they can act as attractants or repellents or alter their orientation ability and day/night cycle [19]– [21]. For this reason, we propose using infrared cameras to record the animal to minimise alteration of behaviours.

Another challenge regards the synchronisation of force and video recordings. To detect the force with respect to the step of an insect, video recordings need to be synchronised with force recording. So a reference point is required to synchronise time stamps accurately. Furthermore, the sampling rate can be crucial when comparing video and force recordings. So, similar sample rate of both recordings can simplify the process.

We consider various parts of the set-up that can cause distortion on recorded images. Lens of cameras, especially wide lenses, can be the first source of distortion due to their characteristics. This type of distortion can be managed reviewing the specifications of lenses.

Prisms, mirrors, or arena parts can also impose a distortion on the recorded image. This type of distortion can be characterised using defined and specific geometrical shapes. For instance, a small ball can be recorded outside of the arena and then within the arena. Both images can be compared, and the effect of all parts characterised.

Table 2. Proposed general improvements of the force plate, the arena and the video recording system and adjustments proposed to adapt the device for smaller insect measurements.

Improvements		**Pros**	**Cons**
Force plate	Calibration with actuator	No human error, better control over the magnitude of the applied force.	Setting up the actuators above the micro-force plate is challenging and time-consuming. Actuators are required to be fully characterised prior to use.

	Optical fibres as alternative to strain gauges	Longer life time preserving the accuracy	Difficulties in building the set-up and reduction of the compliance
	Non-linear fitting method	Account for the nonlinearity in the system by using existing nonlinear fitting models	Fully populated K matrix, more computation for fitting coefficients.
	Resin material	Resin 3D printing gives higher resolution and accuracy compared to other materials	Manufacturing, quality check of the final product
Arena	Washable	Exclude pheromone bias	
	More sensors	More chances for the insect to step on the sensor, reduced experiment time	More complicated design
	Adjustable size	Possibility to use the same arena for different insects' size	More complicated design
Signal	Hardware filters	Background noise reduction, better quality signal	Possibility of altering the signal with filtering
Video recording	Testing for distortion from lenses, prisms, or arena walls	Increases the accuracy of kinematic measurements for very small insects	Time consuming
	Infrared camera	No alteration of the behaviour caused by artificial lights	
Adjustments for usage with small insects		**Pros**	**Cons**
Force plate	Beams: length increase up to 20mm, thickness decrease to less than 0.6 mm	Improvement of force sensitivity and threshold of the force measurement	Difficulties in manufacturing thin slender beams
	Smaller sensor surface ($2mm^2$)	Compatible with smaller insects (based on step span ⅓ of body length)	Less chances for the insect to step on it
Arena	Reduced walkable area	More chances for the insect of stepping on the sensor, reduced time for the bioassays	
	Reduced bending space (gap between the plate and the arena)	The insect cannot enter inside the device	If too narrow the beams do not bend correctly

5. Conclusions

This study underlies the importance of multidisciplinary research. The biological and engineering approach combined allowed us to identify challenges and difficulties of a device developed for the investigation of animals walking strategies. We considered the accuracy of measurements obtained from the system, but also the reliability of the insect behaviour, proposing a number of solutions for the improvement of the experimental protocol.

Additionally, we verified the linearity of force measurement and sensitivity of the device in a force range one order of magnitude lower than that previously investigated, allowing the study of insects with a lighter footfall. This would enable research into identifying new locomotion strategies and possible communication modalities that can be applied in the field of micro robotics, pest management or for ecosystem biodiversity studies.

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