Studies on the banked-turn of Coleopteran flight and electrical stimulation for wing oscillation and foreleg motion to elicit take-off and turning in flight

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**NANYANG
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SINGAPORE

**STUDIES ON THE BANKED-TURN OF COLEOPTERAN
FLIGHT AND ELECTRICAL STIMULATION FOR WING
OSCILLATION AND FORELEG MOTION TO ELICIT
TAKE-OFF AND TURNING IN FLIGHT**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

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Summary

The flight behaviors of insects have been extensively studied for a long time. The idea of making use of natural insects to help human beings is attractive to a lot of researchers. With the continuing development of electronic devices and low-power wireless communication systems, many insect-body-mountable devices have been applied to measure the intrinsic insect behaviors, such as inertia measurement and extracellular recording. Some researchers even successfully applied extrinsic electrical stimulations on insects with tiny wireless stimulators. The appearance of these wireless devices inspired our research and extended our approach from tethered experiments to free flights. Specifically, my focuses are on the stimulated flight initiation of insects, the natural features of banked-turn in insect flight and the roles of foreleg motions in flight control. The result of flight initiation experiment demonstrated that it was reliable to initiate flight on beetle (*Mecynorrhina torquata*, Coleopteran) by electrically stimulating the dorsal longitudinal muscles (DLMs), indirect flight muscles that oscillate the wings. A high success rate with rapid response time on flight initiation was achieved by DLM stimulation. In the measurement of flight banked-turn, a MEMS inertia-measurement-unit was stuck on the pronotum of beetle. The results verified that the yaw angular velocity and body roll angle were highly correlated and the values of yaw angular velocity and roll angle followed a linear relationship. The analysis on foreleg motion revealed that the clockwise and counterclockwise swings of both forelegs were actively induced by beetle itself to deflect the flight course and balance the perturbation. Moreover, we believe that the effects of forelegs in flight should be attributed to their relatively large angular momentum.

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Abbreviation List

Abbreviation	Description
3Ax	Third axillary sclerite
ADC	Analog to digital converter
AHRS	Attitude and heading reference systems
ANOVA	Analysis of variance
AVA	Agri-Food and Veterinary Authority of Singapore
BSM	Basalar muscle
CCW	Counterclockwise
CI	Confidence interval
CW	Clockwise
DARPA	United States Defense Advanced Research Projects Agency
DLM	Dorsal longitudinal muscle
DVM	Dorso-ventral muscle
EMG	Electromyography
FES	Functional electrical stimulation
fps	Frames per second
FWMAV	Flapping-wing micro air vehicle
GINA	Guidance and inertial navigation assistant
IMU	Inertia measurement unit
INS	Inertial navigation system
LED	Light-emitting diode
MAV	Micro air vehicle
MEMS	Micro-electromechanical systems
NACLAR	National Advisory Committee for Laboratory Animal Research
PCB	Printed circuit board
RF	Radio frequency
RMSE	Root mean square error
SBM	Subalar muscle
SD	Standard deviation

Chapter 1 : Introduction

1.1 Background

1.1.1 Insect Flight

The insects reveal excellent agility and maneuverability in air, which has inspired human beings more than any other animal behavior [1]. The flight is important for a large number of activities among winged insects, and the morphology features have been well developed to adapt their habits. The flapping flight needs the collaboration of wings to produce aerodynamic forces, muscles to drive the wings, and a nervous system to modulate the powers of the muscles, which makes it apparently different from the fixed-wing aircrafts [2]. The efficient flight muscles can offer high maneuverability and long duration for flight [3]. Normally, the flight muscles of insects are divided into a few large power muscles and many small steering muscles. The power muscles contract cyclically with the wing beat for the sake of generating lift and thrust whereas the steering muscles control the force transmission between the power muscles and the wings through the complicated wing hinge [4, 5]. The wing hinge contained several mobile hardened cuticles lying between the wing and the thorax, which can be manipulated to change the wing stroke and thus change the aerodynamic force. Then flight maneuvers can be arose by generating asymmetric forces between the left and right wings.

1.1.2 Flapping-Wing Micro Air Vehicle

The micro air vehicle (MAV) was defined by the United States Defense Advanced Research Projects Agency (DARPA) as a flyer whose mass is less than 100 g with the maximum constrained size of 15 cm. MAVs are expected to be used for exploring the hazardous environment, search and rescue mission, and agriculture assisting [6]. Among

the MAVs, flapping-wing devices are of great interest as they have been exemplified by the natural counterparts. With the progress in understanding the control, stability and aerodynamics of wing flapping, researchers have already developed and demonstrated flapping-wing micro air vehicle (FWMAV) which is capable of hovering in air (similar to large insects) [7, 8]. However, the scaling down of body size led to the extreme reduction in payload capability, which means the FWMAV still needs to be tethered for power, sensing and control. Despite of these limitations, the mimicking of flying insects to develop new type of FWMAV still kept developing. In 2002, James Delaurier and his students, from University of Toronto, introduced the first radio control FWMAV that was able to hover [6]. Robert J. Wood's team demonstrated the first take-off of "RoboBee" in 2007 and recently hovering, perching and basic flight maneuvers were developed as well [9, 10]. Phan, Kang [8] successfully applied feedback control on a 21 g insect mimicking FWMAV. We believe more and more new achievements will be continuously reported to promote the development of the biological inspired insect-scale FWMAV.

1.1.3 Insect-Machine Hybrid Flying Robot

The idea of the insect-machine hybrid flying robot was put forward in the recent decade when MEMS and electronic technologies enabled such devices for controlling the insect in untethered condition. Unlike the industrially manufactured artificial MAVs, the insect's natural flying mechanism would become a highly flexible flying platform by attaching tiny electronic devices onto the insect. Moreover, the hybrid robot could solve the limitation of power source because the power for flapping was from the internal source of insect itself rather than the external battery. Thus, insects can be considered for developing an ultra-low power MAV that has a perfect flight maneuver. The earliest

devices used in insect flight came out in the early 90s for measuring the muscle potentials [11, 12]. The miniaturization of the electronics enabled certain approaches for stimulating the insect in free flight, which made the insect itself a promising candidate for controllable living MAV [13, 14]. In the future, the fuel cell may be implemented to replace the commercial battery or adding a secondary backup for charging the battery because the fuel cell can use glucose as fuel which can be directly extracted from insect liquid.

1.2 Motivation

Researchers have long been interested in reconstructing natural insects into steerable robots or vehicles. Owing to recent advances in nano/micro manufacturing, data processing, and anatomical and physiological biology, we can now stimulate living insects to induce user-desired motor actions and behaviors. Some preliminary works on flying insect-machine hybrid system have been done on moths and beetles in flight because of their relatively larger body size and stronger payload capability. In this thesis, the insect-machine hybrid system uses beetle as the platform because beetle showed more advantage in payload capability and life cycle. Specifically, beetle can carry up to 30% of its weight and lives for more than 3 months in mature stage whereas moth can only survive for a few weeks with much smaller payload [13-16]. The insect flight involves multiple muscles to drive the wings, and a nerve system to modulate the powers of the muscles. The stimulation of nerve system is too difficult as it is hard to locate the nerves as well as to implant the electrodes. As a comparison, the stimulation of flight muscles is far more feasible according to their size and morphology. However, the control of flying insect-machine hybrid system now requires precise protocol in terms of the targeting actuators and the stimulation parameters. It is possible that the

stimulation of target muscle might affect the nearby muscles and the deficiency in nerve or muscle would violate the flight control and lead to unpredicted behaviors on beetle. In order to achieve the precise control on freely flying beetles, a protocol for stimulating the muscles must be well considered and designed.

1.3 Objective and Scope

To achieve the insect-machine hybrid flying robot, the method for flight initiation should be well designed at the first stage. It is known that the previously reported method for flight initiation on beetle could induce permanent damage. Thus, a reliable and safe method needs to be proposed.

Although insect flight has been long studied, many studies were carried out only on the tethered insects and the roles of many individual parts are still awaiting discovery in free flights because of the limitation of appropriate motion capture system and insect-body-mountable wireless device. With a motion capture system, the position of small objects can be accurately detected in a relatively large flying area. In addition, the wireless device is required to measure or stimulate the flying insect. Thus, we would like to demonstrate miniature devices that can be mounted on insects and reveal the characteristics of insect flight in untethered condition.

Therefore, I will focus on the following objectives in my PhD thesis:

- Achieving the flight initiation of beetle by electrically stimulating one or more muscles with high success rate ($> 85\%$) and the repeatability (retain normal flight steering after 10 stimulations). Meanwhile, the damage caused by the stimulation should be mild enough for the beetle.

- Designing and manufacturing a wearable inertial measurement unit (IMU) for sensing and recording beetle body attitude angles and flight accelerations wirelessly in free flight. The banked-turn in flight can be quantitatively evaluated.
- Studying the role of the large forelegs in flight and demonstrating it with a miniaturized neuromuscular stimulating backpack and a 3D motion capture system.

1.4 Significance

- Provide deeper understanding of an individual muscle (dorsal longitudinal muscle) in flight initiation. This study will be of great practical use in initiating hybrid MAVs.
- Demonstrate the body attitudes during a flight turning and provide detailed relationship between the body roll angle and the yaw angular velocity.
- Reveal the effects of foreleg motions in flight turnings and verify the roles of body movements in flight control.

1.5 Organization of the Thesis

Chapter 1 gives a brief introduction, motivation, objectives and scopes as well as the significances of the project. The organization of the report is also included.

Chapter 2 presents the literature review of the project. It includes the introduction of insect flight, flight initiation and landing, and the stimulations for insect flight study as well as the wearable miniature devices.

Chapter 3 describes the experimental procedures for insect flight study.

Chapter 4 discusses and evaluates the flight initiation induced by electrical muscular stimulation.

Chapter 5 discusses and evaluates the characteristics of banked-turn in beetle flight.

Chapter 6 discusses and evaluates the functions of forelegs in flight control.

Chapter 7 presents the conclusion based on current study results and summarizes the remaining works in the future on insect-machine hybrid system.

Chapter 2 : Literature Review

2.1 Insect Flight

The extraordinary maneuverability of flying insects is mainly because of their ability to manipulate the aerodynamic forces in unsteady airflows from wing flapping, and a well-developed sensory and neuromotor system. The extraordinary ability of insects has attracted many biologists to keep investigating their flight principles and mechanisms since the appearance of the first ornithopter by Alexander Lippisch in late of 1920s [17]. Based on their endeavors, the study of insect flight has experienced a remarkable development from the extrinsic observation of wing structures to the intrinsic investigation of muscular and nervous activities.

2.1.1: Structure of Flying Insect

Figure 2.1. Dorsal view of body segments of a Coleopteran [18].

The body of winged insect is generally divided into three tagmas, namely the head, thorax and abdomen (Figure 2.1).

The head of insect is the smallest among the three tagmas, which makes it easy to distinguish. The head is heavily sclerotized into a hard epicranium and is flexibly connected to the thorax through a soft cervix, or neck. The flexibility of the head is achieved by the cervix.

The thorax consists of three separated segments, namely the prothorax, mesothorax and metathorax. From the dorsal view, the prothorax can be visually observed whereas the mesothorax and metathorax are hidden by two large elytra, arising from the second thoracic segment. Each segment of insect's thorax bears a pair of legs. Specifically, the prothorax, first segment of thorax, bears the prothoracic legs, or forelegs. The second segment is the mesothorax with its mesothoracic legs, or midlegs. The mesothorax and the prothorax are connected via a flexible articulation. The third segment, the metathorax, rigidly follows behind the mesothorax. The metathoracic legs, or hindlegs, are stretched out from this segment.

The typical insect leg consists of six articles. The cylindrical coxa is large in size and locates at the bottom of the sternite of the thoracic segment, which partly embedded into a cylindrical recess of the sternite. A small anatomical article at the distal end of the coxa is the trochanter, which is a flexible mechanism connecting the femur to the coxa. The femur is a long and thick article extending distally from the trochanter, which has a similar length with the coxa. The article following the femur is the tibia. The distal end of tibia is called tarsus and the tarsus is divided into several smaller articles arrayed in sequence. Those small articles are the

tarsomeres. The distal tarsomere bears two long tarsal claws. The tarsal claws and the accessories are the sixth leg article, which are also known as the pretarsus.

The mesothorax and metathorax together can be known as the pterothorax. They are named so because the two wing pairs, forewings and hindwings, are bore here. The forewings of Coleopteran are heavy sclerotized into elytra, which is mainly for protecting the delicate hindwings. The hindwings are much longer than the elytra and consequently must be folded to fit in the space below the elytra.

The abdomen is easily seen in the ventral view whereas covered by the elytra and wings in the dorsal view. The abdomen of beetle consists of several visible segments, which are covered by sternites. On the upper surface of abdomen, there are sclerified strips of dark cuticles extending transversely named the tergites.

2.1.2: Morphology of Flight Apparatus

The structure of insect wings

Figure 2.2. Articulation of the wing with thorax [5].

Apart from the vertebrates, insects are the only group of animals which have the ability to fly. The aerodynamic forces during the insect flight are generated by their wings. The insect wings are adult outgrowths of the insect exoskeleton which evolve from the gill-like appendages [19]. The wings locate at the mesothorax and metathorax and connect to the thorax at three points with various forms of axillary sclerites which were defined by Snodgrass [20] (Figure 2.2). The movements of these sclerites are controlled by the flight muscles and function on the wings [5, 21]. Specifically, the first sclerite hinges on the anterior notal process horizontally. The second sclerite connects with both dorsal and ventral membranes and articulates with first sclerite and pleural wing process. The third sclerite hinges on the second sclerite and the posterior notal process vertically. The basalar and subalar sclerites articulate with the pleural wing process in anterior and posterior position, and both of them locate beneath the wing base. Moreover, the wings are divided into areas by fold-lines and flexion-lines to enhance both the rigidity and the flexibility in the flexion or folding. Generally speaking, there are four areas on the insect wings, namely the remigium, the anal area (vannus), the jugal area and the axillary area.

Wings consist of two layers of cuticular membrane with a framework of veins embedded between them. The veins are sclerotized and provide a strengthening structure to the wings, especially the longitudinal veins, the venation (Figure 2.3). According to current dogma, the archdictyon contained 6 to 8 longitudinal vein, which are named by a system devised by John Comstock and George Needham [22]. There is a nerve and a trachea within the major veins, and hemolymph can flow into the veins as the cavities of veins are connected to the hemocoel [5].

Figure 2.3. The venation of hind wing of *Dorcus titanus platymelus*, where C is costa, MP is media posterior, Cu is cubitus, and AP is anal posterior [23].

The areas separated by the veins are called cells. Specifically, the areas surrounded by veins only are called closed cell and the areas surrounded by veins and wing margin are called open cell. Moreover, the name of each cell is given by the vein on its anterior side.

Flight muscles

The pterothorax is the basement of insect wings and it is limited by the tergum at the top and sternum at the bottom. The wings are connected to the pterothorax at the distinct sclerites (Figure 2.2). The movements of the sclerites are controlled by the flight muscles, which were encircled into the pterothorax. In general, the flight muscles of insects need to take up at least 12% to 16% of the total body mass [24].

Figure 2.4. Representative spatial positions of direct flight muscles and indirect flight muscles [25].

According to the spatial positions and connections, the flight muscles are cataloged into direct flight muscles and indirect flight muscles (Figure 2.4). The two kinds of muscles reveal different functions in the flight mechanism. The direct flight muscles insert on the wing base or on the cuticular patches in the wing articulation, which can directly cause effect on the wing movement, whereas the indirect muscles induce wing movements indirectly by changing the position and shape of the thorax and the hinges on the thorax [20, 21, 25-30].

Dorso-ventral muscles (DVMs) and dorsal longitudinal muscles (DLMs) are the representative main indirect flight muscles (Figure 2.5a). They serve as the main powering mechanism for flapping the wings by contracting antagonistically. The DVM lies between the sternum and the tergum vertically, so its contraction can

compress the thorax vertically which helps lift the wing upwards. The DLM lies along the body longitudinal direction from the posterior cuticle to the anterior cuticle, so its contraction can expand the thorax horizontally which makes the wing extend [25, 28-31].

Direct flight muscles (Figure 2.5b) directly link to the sclerites of wing base through the apodema or ligament. Specifically, the basalar and subalar muscles start from the pleuron and hindleg coxa and eventually connect to the apodema of the basalar sclerite and subalar sclerite, respectively. The functions of these two muscles are usually known as depressing and twisting the wings [4, 32, 33]. Malamud [34] found the locust employed the metathoracic second tergocoxal muscle as the wing levator and coxal remoter. In addition, one muscle, inserted from the pleuron to the third axillary sclerite through a tendon, helps folding the anal part of the wing and flexes the wing backwards [5, 35].

Figure 2.5. The configuration and connection of flight muscles of a locust. (a) Indirect flight muscles. (b) Direct flight muscles [5].

2.1.3: Generation of Flight Force

Figure 2.6. Simplified demonstration of muscle contractions for wing flapping.

(a) The indirect dorso-ventral muscles cause wing elevation; (b) direct basalar muscles cause depression, such as in dragonfly (Odonota). (c) The elevation of the wing is produced by the dorso-ventral muscle; (d) the depression of the wing is produced by the indirect dorsal longitudinal muscle, such as in fly (Drosophila). The side view of (e) the contraction of dorso-ventral muscle depressed the tergum and (f) the contraction of dorsal longitudinal muscle [5].

The flight force is produced by the upward and downward flapping of the wings. While the contraction of DVM pulls the tergum and its articulation with the wing down to move the wing upward [5, 25], the downward movement is more complicated. The downward wing flapping can be produced in two different ways. One is to use the direct basalar muscle for wing depression (Figure 2.6b) and the other one is to use the indirect dorsal longitudinal muscle for wing depression (Figure 2.6d).

In large insects like the Ephemeroptera and Odonata, the DVM of insert is hinged at the tergum and its contraction pulls the tergum downwards. As the tergum is connected with the wing base, a downward movement of the tergum presses the wing base down and thus lifts the wing up, which is like rowing through the air. When the wing is elevated by the DVM, a direct flight muscle, basalar muscle, is activated and the DVM turns to relax. Since the basalar muscle is mechanically coupled to the wing via a tendon at the basalar sclerite, the contraction of the basalar muscle rotates the wing hinge downward [5].

In other insects, the orthogonal configuration and the alternating contraction of the DLMs and DVMs are the key mechanisms in generating wing oscillation. As shown in Figure 2.6 c-f, the upward and downward motions of the wings are well explained. The relaxation of DLM and the contraction of DVM pull the tergum as well as its articulation at the wing base downwards and inwards which will lift the wing upwards. The relaxation of DVM and the contraction of DLM push the tergum as well as its articulation at the wing base upwards and outwards which will depress the wing downwards [5, 21, 25]. In this configuration, the basalar muscle is not

used for generating wing depression but contracts to allow the wing to flap through a larger wing stroke [4, 33].

Figure 2.7. Wing thrust (upper trace) during tethered flight and associated muscle action potentials (lower trace) from an asynchronous muscle and a synchronous muscle [36].

The flight muscles for high-frequency operation are divided into two categories, the asynchronous (fibrillar) muscles and the synchronous (nonfibrillar) muscles, based on the neural control modes [25, 30, 36, 37]. Synchronous muscles are those in which there is always a muscle potential change evoked by neural activity under each contraction. The asynchronous muscles can contract in an oscillatory fashion without the congruence between muscle potentials and mechanical contractions when activated (Figure 2.7). Both synchronous and asynchronous muscles are found in insect flight muscles. The asynchronous muscles evolve from the synchronous

muscles and they represent a design breakthrough as they are more efficient and powerful in generating high-frequency operations. It is mainly because the metabolic expenditures associated with calcium cycling are much lower in asynchronous muscles than in synchronous muscles [25, 36].

2.1.4: Flight Control and Flight Steering

The flight control is mostly achieved by the flexible wings which can change shape while rotating around the wing hinge. In the study of wing kinematics, the wingbeat frequency, stroke plane angle, stroke amplitude and wing rotation are generally used [5].

Figure 2.8. (a) Wing stroke plane angle. (b) Stroke amplitude. (c) Wing twisting during flapping [5].

Wing beat frequency of insect varies from 10 Hz to several hundred Hz and negatively correlates with body mass. The change of thorax temperature can change

the wingbeat frequency that regulates the aerodynamic power output. The changes in wingbeat frequency correlate with the changes in stroke amplitude in most insect species. As the frequencies of the wings on both sides are always same, it cannot play a role in generating asymmetrical lateral force [5].

The stroke plane angle is defined as the inclination of the stroke plane, relative either to the longitudinal axis of the insect body or to the horizontal (Figure 2.8a). The stroke plane tends to incline forward in the fast flight. In the insects with low wing beat frequencies, this angle remains almost constant in flight. The differences in the stroke plane angles of the left and right wings can induce uneven lateral forces [5, 27, 33, 38].

Stroke amplitude is the angle defined by the top and bottom limit of the wing stroke cycle within the stroke plane (Figure 2.8b). The amplitudes of the flapping wings typically fall into the range of 70° to 130°. The stroke amplitude is important in regulating the flight power output as its value varies greatly in flight. The larger amplitude the wing flaps, the greater force the wing generates. The differences in stroke amplitudes of the left and right wings can be used for flight steering and the larger stroke amplitude drives the insect to turn towards the contralateral side [27, 33, 38-41].

During flapping, the wing imposes rapid rotations about the long axis of the wing (Figure 2.8c). The pronation is elicited when the leading edge of the wing rotates downward at the beginning of the stroke. The motion of rotating the leading edge of the wing upward is named as supination. The rotation of the wing is produced by differential action of the basalar and the subalar muscles inserted at the wing base.

The rapid rotation may be a way to increase the aerodynamic lift. The angle of attack is regulated by changing the pronation and supination of the wing that leads to change in aerodynamic forces. The increase of pronation serves to reduce the the angle of attack, which will induce an ipsilateral turning [27, 38, 39].

Figure 2.9. Different leading-edge vortex topologies are possible in flight, namely (a) extends across the thorax, (b) attaches at the base of each wing, and (c) forms a separate horseshoe-shaped vortex system on both wings [42].

Aerodynamics is an important topic in the study of insect flight with various practical applications and simulation analysis for predicting the generated aerodynamic forces during wing flapping [27, 43-46]. It not only helps to improve the knowledge of flight control of insect but also inspires the further development on FWMAVs.

When the insect flaps their wings, the aerodynamics of flapping differs in two important ways. One is the separated flow which means the air tends to become entrained in a swirling vortex over the upper surface of the wing; the other one is the unsteady flow which means the separated flow over a flapping wing varies continually (Figure 2.9). It is supported that insects extensively use the unsteady separated flow mechanism to generate the aerodynamic forces. The most ubiquitous and significant separated flow should be the leading-edge vortex. The leading edge vortex causes the pressure reduction on top of the wing that leads to an upward suction force known as vortex lift [27, 42, 43, 46, 47].

Apart from the wings, some body parts, like heads, legs and abdomens, are involved in the flight control as well. Even though they cannot steer the flight as powerful as the wings, they reveal their contributions in yaw correcting and perturbation balancing.

Head movement was found in the correctional flight maneuvers [48-50]. The head was not directly functioned on the flight manipulation but affects the flight muscles as the sensory input [49]. The head rolling was found regulating the strength of flight steering on locusts and the neck flexes drove the body to orient to the head [50].

Figure 2.10. The representative deflection of hindleg in flight steerage [51].

As to leg motions, the motions of hindleg were the mostly studied [51-58]. A study of the hindleg of cricket drew a conclusion that the hindleg motions were induced to impede the wing trajectory and shorten the response time of turning [55]. Lorez [54] concluded that the left or right extension of hinglegs of locust in flight steering was mainly for increasing the air drag like a rudder. Another research on the role of hindlegs of locust proposed that apart from air drag, the shift of the center of mass and the altered moments of inertia could also be the explanation of hindleg usage (Figure 2.10) [51]. Meanwhile, the bees were proved to use their hindlegs to stabilize the body orientation in flight [57, 58]. By altering the moment of inertia of a flying insect, the flight stability can be increased while the flight efficiency is decreased.

Figure 2.11. The vertical abdominal movement of a flying honeybee [59].

More observations were reported on the abdominal deformation in flight steerage [52, 53, 56, 59-63]. The study on abdomen deformation suggested that the lateral or vertical movements of the abdomen could be elicited by the sensing organs on the head by demonstrating that both visual stimulation and wind stimulation could induce the abdominal responses (Figure 2.11) [53, 60, 63]. A researcher studied the correlation between abdomen and wing flapping and in his paper he proposed a thought that the big inertial of abdomen may be the reason for the deflection [56]. However, in another paper studying the flies, the independence of abdomen steering were proved and the roles of abdomen deflection were attributed to the air drag and the torque generated by gravity [62].

2.1.5: Studies on Coleopteran Flight

The Coleopteran is the largest group of all animal orders, making up at least 40% of all insects, and new species are still frequently discovered. A kind of Coleopteran

(*Mecynorrhina torquata*) was used in my research as it has relatively larger body size and higher payload capacity.

The toughened forewings, or elytra, of Coleopteran meet down the body midline and cover the larger membranous hindwings, which are folded lengthwise and crosswise underneath at rest [5]. Coleopterans spread their elytra at a dihedral angle and do not keep them still in flight, which are swung at low amplitudes in a same frequency with hindwings. The effect of the elytra in sustaining the flight is small, but not negligible because elytra may serve to improve the airflow for the hindwings [64]. Meanwhile, it is also found that the elytra were effective in generating uneven lift by placing them in different angles [65].

Similar with most flying insects, the flapping of the hindwings of Coleopteran is produced by the indirect flight muscles, DLMs and DVMs, and the fibrillar basalar muscles are widely used in the downstroke as well. For most Coleopteran, the flight initiation needs to occur within certain temperatures, which can be prepared by muscular activities inside the thorax [66].

2.2: Flight Initiation and Landing

2.2.1: Flight Initiation

The flight initiation plays a quite important role as it is the first step of all flights. The pre-flight temperatures of insects have been well studied, including extrinsic environmental temperatures and intrinsic thoracic temperatures. It is found that temperature is an important extrinsic factor for the take-off in nature. A relatively higher environmental temperature, which is usually between 25 °C and 35 °C, is more suitable for the flight initiation [67-69]. Moreover, it is found that the thoracic

temperature is usually clearly higher than the environmental temperature before flight. The studies on intrinsic muscular activities prove that some insects, moths and beetles for instance, purposely rub their muscles or organs in the thorax to increase their thoracic temperatures before flight [66, 70].

Figure 2.12. The opening of elytra and hindwings during non-jumping flight initiation of a *Rhinoceros* beetle [71].

For the take-off behaviors, most insects, like flies, bugs and cicadas, prefer jumping into the air before flapping the wings [72-74], whereas some other insects, such as the *Rhinoceros* beetles, choose the non-jumping take off [71]. The jumping is a common way in initiating flight in flying animals because the jumping from the hind legs gives a rapid initial velocity and a sufficient space for wing beat. In the jumping, the air flow to the head and the loss of tarsal contact are consequently set up [75]. However, a study on *Rhinoceros* beetle reported the non-jumping take off, which showed that the beetle had flapped the wings three times before the legs lost contact with ground and the force for the initiation was totally from the hind wings (Figure 2.12).

Figure 2.13. The optic lobe stimulation in beetle [76].

(a) The electrodes were implanted into the optic lobes of the beetles. (b) When the optic lobes were applied the train of 100Hz, the beetle initiated the flight while the beetle stopped flying with a single pulse.

Apart from the observations of natural take off, several stimulating methods for flight initiation have been proposed in insect study. Specifically, a wing stimulus to the head coupled with a loss of contact of the tarsi with the ground is the mostly used method to evoke flight [75]. Moths have been initiated by applying electrical signals to the brain or antenna [77, 78]. The applied electrical signals could not only initiate the wing beat but also alter the flight directions. Flight of cockroach can be initiated in wind puffs after the injection of some chemical, octopamine [79]. The chemical can activate the wing-sensitive neurons in cockroach and apparently increase the rate of take-off in the wind puffs. Beetles have been initiated by two different methods, namely electrical stimulation [65, 76] and thermal stimulation [80]. The electrical stimulation for initiating flight was exerted on the optic lobes of beetle with pulse signals (Figure 2.13); the thermal stimulation was applied by heat coils at the antennal base. The electrical stimulation of optic lobes could start the flight when on ground through 100 Hz pulses and stop the flight when in air through a 1-s single pulse. The thermal stimulation cannot stop the flight after the initiation, but it can change the flight direction by heating one side of the antenna.

2.2.2: Flight Landing

Typically, for an insect, landing consists of a deceleration in flight velocity and extension of one or all pairs of legs until the surface is contacted, whereupon the beating of the wings slows or stops and the legs are brought into contact with the surface [81] (Figure 2.14). As proposed by Goodmann [81], the wingbeat of a fly stopped as soon as the first and second pairs of legs touched the ground or substrate. The landing behavior can be recognized by lowering the legs, which should be evoked by the vision inputs from the compound eyes. The moment at which the legs

are lowered is not based upon the estimation by the fly of the distance between itself and the surface it is approaching. A normal landing response can be evoked merely by decreasing the light intensity of the surroundings without any movement occurring in the visual field [64, 81].

Figure 2.14. The leg motions in the landing of a tethered fly induced by an approaching disk [81].

In the landing of bumblebees, body and head have a relatively constant orientation at the moment of leg extension and the legs are extended at a distance of 8 mm from the landing substrate. It is found that the duration of the hover phase stayed more or less the same throughout all landing conditions [82]. As a close relative, the honeybees reveal similar final moments of landing. The honeybees enter a stable hover phase at a constant distance from the landing surface, independent from the tilt of the surface. However, the increased tilt progressively inclines the body streamline and elevates the antennae, indicating the capability of bee's visual system in estimating the tilt of the surface. Touchdown is initiated by extending the hind legs when landing on horizontal or sloping surfaces, and the front legs or antennae when landing on vertical surfaces [83].

2.3: Stimulations for Insect Flight Study

2.3.1: Optomotor Stimulation

Optomotor stimulation, or visual stimulation, is a method that has been widely used in insect flight study for long time (Figure 2.15). Mostly, the insect was tethered in a flight chamber with optic flow patterns projected [35, 48, 50, 53, 59, 62, 84]. For small insects like the flies, free flight was adopted in a flight arena [43, 85, 86]. When the visual patterns are applied, it evokes the insect's optomotor organs to generate the neural excitations to the actuators for performing required functions. Meanwhile, measurements on the insect are usually conducted at the same time for recording the body responses, wing kinetics or neuromuscular activities. It is suitable and efficient for studying the flight maneuvers of most insects.

Figure 2.15. Flight arena for visual stimulation and insect model for calculation [84].

(a) The sensor and controller processing blocks of the visual–abdominal reflex were characterized by presenting visual pitch rotations to tethered moths in a cylindrical LED arena. (b) The dynamics of the plant were derived using a simple physical model of the moth composed of two masses, corresponding to the abdomen and the thorax, connected by a hinge joint.

Both tethered experiments and free flights were conducted on the study of flies. The roles of abdomen and leg movements were analyzed according to the optomotor stimulation in the tethered experiments [53, 62]. With the high-speed cameras, the wing kinetics in free flight could be recorded within the flight arena [43, 85, 86]. As to other insects, the visual-abdominal reflex of moth was studied and modeled in a vertical cylindrical LED arena (Figure 2.15) [84] and the abdomen movement of bee was elicited by horizontal planar monitors [59]. Rotational and horizontal visual patterns were used to observe the head movements in locust flight [48, 50]. The neuromuscular activities in the visually induced left-right flight turnings were recorded on beetles [35].

2.3.2: Electrical Nerve Stimulation

The electrical signals onto the nervous system of the insect like brain, optic lobes, central nervous system, and nerve branch can be applied to elicit desired behavior on the targets. It is found that the stimulation of optic lobes of beetle could initiate and terminate the flight [65, 76]. The central nervous system was excited and the command to initiate the flight was generated when pulse signals at 100 Hz were applied on the optic lobes. The beetle stopped flying when a single long pulse was applied at the same position. The nerve stimulation was used on moth flight manipulation as well. It is found that the electrical pulse signals at 20 Hz onto the antennal lobe could cause wing flapping and flight initiation on a resting moth [77, 78]. As the movement of abdomen in moth flight was proved effective in balancing the flight, nerve stimulation on the nerve cord located inside the abdomen was carried out for flight control (Figure 2.16) [87]. The nerve cord was implanted by a multisite flexible split-ring electrode during the pupae stage. Electrical stimulation could be transmitted via the electrode to evoke multidirectional and graded abdominal motions and the motions were confirmed effective in flight directional control in a loosely tethered condition.

Figure 2.16. Electrical stimulation of abdomen nerve cord in moth [87].

(a) Insertion process of the flexible split-ring electrode into the abdomen nerve cord.

(b) The cyborg moth with the electrode implanted in pupae and adult stage. (c) The

detailed view of the implanted electrode and the abdomen nerve cord.

2.3.3: Electrical Muscle Stimulation

Directly applying the electrical signals to muscle tissues is proposed as a promising method to induce contractions on the target muscle. This stimulation method has been widely implemented on the study of insect flight control by now. As the flight muscles are directly involved in the flight control, the stimulation of flight muscles

could help generate some flight maneuvers of the insect. It is found that the stimulation of the beetle's basalar muscle could induce the contralateral flight turning whereas the stimulation of the beetle's 3Ax muscle could induce the ipsilateral flight turning (Figure 2.17) [35, 76]. It is also found the stimulation of 3Ax muscle revealed graded turning effect as a function of the signal frequencies from 40 Hz to 90 Hz (Figure 2.17b). In moth, the indirect flight muscles, DVMs and DLMs, were stimulated to produce the flapping of the wings [14, 16]. Meanwhile, the stimulation of antennal muscles of moth could move the direction of antenna and thus affected the mechanosensory system of moth. As a result, the moth changed its flight path [15]. Similar as the antennal muscles, neck muscles of moth control the head movement, which is also involved in the flight control. The stimulation of neck muscles could change the yaw direction in flight [77].

Free flight experiments

Figure 2.17. Stimulation of the 3Ax muscle of beetle in free flight [35].

(a) Electrical stimulation of the left 3Ax muscle for left turn and the right 3Ax muscle for right turn in sequence produced a zigzag flight path. (b) Lateral force induced by the electrical stimulation of 3Ax muscle was graded as a function of stimulus frequency.

2.3.4: Other Forms of Stimulations

Apart from the aforementioned stimulation methods, various other forms of stimulation have been demonstrated in the insect flight study. Some of these methods directly functioned on the insect body, or even inside the insect body, while some others stimulated the insect externally by inducing the mechanosensory reactions in flight. Specifically, the chemical dose injection in moth reduced the flight power output by around 50% because the injected chemical could overstimulate the central nervous system near the DLM [78]. The injection of octopamine to the abdominal ganglion or the metathoracic ganglion of cockroach apparently reduced the threshold in wing velocity for flight initiation [79]. Thermal stimulation was used at the antennal base of beetles, which exploited the natural fire aversion behavior (Figure 2.18a) [80]. The heat generated by a microthermal actuator induced flight initiation and directional control on beetles. Direct mechanical intervene on body orientations in flight were used on the study of head rolling under roll intervene on wasps (Figure 2.18b) [88] and abdomen flexion under pitch intervene on moths [63]. Moreover, the wind and ultrasonic sound were also applied to trigger the mechanosensory system during insect flight. It is found the cricket moved its contralateral hindleg to impede the wing trajectory when ultrasonic stimulation was generated from one side of body [55]. The observations on abdomen and hindleg movements in locust flight turnings were induced by altering the input wind angles [51, 52, 61].

Figure 2.18. Thermal stimulation on beetle and mechanical stimulation on wasp.

(a) The Ni resistive stimulator was bonded near the antennal base of beetle and the sharpened tip localized the heat stimulation [80]. (b) Wasps were tethered by waxing a strip of cardboard to their thorax and mounted onto the shaft of a servo motor, which was used to rotate the body in roll [88].

2.4: Wearable Miniature Devices for Insect Study

The study of insect flight in tethered condition has been popular for a long history since the beginning of this field. The study insect was fixed on a holder or loosely wired to perform the recording or stimulation (Figure 2.11). The tethered experiment was used for studying the insect flight mechanism with the recording of high speed cameras [59, 65, 81, 89]. The insect physiology could be analyzed as well by recording the neural and muscular activities on a tethered insect, which directly revealed their roles during wing flapping [66, 90, 91]. However, tethered experiments restrict the control of flight attitudes; consequently, the wing kinematics and body postures of tethered insects may deviate from their natural behaviors [92]. Successful demonstrations on insect-body-mountable devices have inspired an alternative approach, the mounting of tiny measuring devices on the insect body, which has gained traction in recent years.

Figure 2.19. (a) Two transmitters made of surface mount electronic components were attached on the lateral prothoracic position of a locust [93]. (b) A moth was mounted with a dual-channel transmitter [94].

The development of electronic device enables the possibility of measuring the muscle potential in untethered condition. The muscle potential recording of the locusts in free flight was introduced from the early 1990s (Figure 2.19a) [11, 12, 93]. The recorded potentials of two flight muscles were synchronized with the wing strokes to distinguish the functions of selected muscle and the interaction with nearby muscle [93]. The muscle potential recording of the moths was further developed in early 2000s (Figure 2.19b) [94-96]. The muscular activities of DLMs and 3Ax muscles were measured during flight and synchronized with the flight trajectory, which compared their excitations between the flight and the rest condition [94].

The advances in miniaturized electronic devices have started a new era on the field of insect flight and the idea of insect-machine hybrid system has come true. The control of insect flight in untethered condition in the recent decade was remarkably

developed [15, 76-78, 80, 87]. In these systems, the exogenous stimulation was applied on the flying insect to trigger and then observe the desired motions of the insect. Moth is the most widely used insect in these studies. The chemical injection was used in moth flight manipulation via a balloon-assisted device to induce a recoverable reduction in flight velocity [78]. It is also proposed in this study that the electrical stimulation of the DVM could greatly elongate the free flight duration. Hinterwirth, Medina [15] demonstrated the flight directional control by stimulating the antennal muscles with a wireless backpack. The neural stimulations were carried out on moths during free flight as well. Specifically, the antennal lobe was electrically stimulated with a balloon-assisted stimulator to initiate, terminate and steer the flight (Figure 2.20a) [77]. A split-ring electrode inserted into a nerve cord in the abdomen of moth could be used to bias the flight path [87]. Moreover, some researchers designed miniature electrical backpack for the study of beetle flight, which could apply multi-channel pulse signals without affecting the natural flight ability [35, 76]. The use of these backpacks successfully demonstrated the steering effects of some flight muscles (Figure 2.20b). The conceptual design on remote microthermal heater was proposed and verified for the flight initiation and directional control of beetle [80].

Figure 2.20. Insect-machine hybrids were made of live insects and electronic devices.

(a) The radio-controlled stimulator was used to control the initiation and cessation of flight in moth by stimulating the brain [77]. (b) The wireless electronic backpack was mounted on the pronotum of beetle to steer the flight via the electrodes implanted into the 3Ax muscles [35].

It can be predicted that the insect-machine hybrid system will lead to a vibrant development in the future. The hybrid system reveals clear advantages compared to artificial MAVs because the insect's natural flying ability will provide a high flexible flying platform and the internal energy of insect will solve the limitation of power for flapping. However, further investigation of insect flight control in untethered condition still faces some technical limitations, such as the small payload capacity, heavy wireless system and lack of long-lasting power source.

Chapter 3 : Experimental Procedures

3.1: Study Animal

Animals used for all experiments in this study were adult *Mecynorrhina torquata* beetles (order Coleopteran; length: 62 ± 8 mm; mass: 8.8 ± 1.9 g), which were bred with commercial beetle jellies twice a week and were kept in pinewood-bedded plastic terrariums (20 cm $\times$ 15 cm $\times$ 15 cm). The temperature of the rearing room was maintained at nearly 25 °C and the humidity in the room was around 60 %. The natural flight ability of every beetle was tested before experiments, which was done under the criterion that intact beetles can fly at least 10 s [35]. The flying ability was commonly tested throughout this study to judge whether beetles can normally fly after we removed elytra or scutellum, blinded folding, implanted electrodes, and electrically stimulated DLM or optic lobes. The use of this animal is permitted by the Agri-Food and Veterinary Authority of Singapore (AVA, HS code: 01069000, Product code: ALV002). Invertebrates, including insects, are exempt from ethics approval for animal experimentation according to the National Advisory Committee for Laboratory Animal Research (NACLAR) guidelines.

3.2: Electrode Implantation

A beetle was firstly anesthetized in a small sealed bag containing CO₂ for 1 minute. The beetle's legs were first immobilized by rubber band so that it could not disturb the implantation. Two tiny holes were pierced through the cuticle above the target muscle by insect pins (enamel-coated #5, Indigo Instruments). A 10-cm segmented Teflon-insulated silver wire (127- μ m uncoated diameter, 178- μ m coated diameter; A-M Systems) was used as an implanted electrode. One side of the silver wire was flamed to remove the insulated layer and then inserted through a tiny hole to the

muscle tissue to the depth of ~4 mm. The two electrodes for each muscle were placed with a distance of 3 - 4 mm. The same implantation sites for each muscle was used and the positional deviation was smaller than 2 mm. Melted beeswax was dripped on the tiny holes because beeswax could quickly solidify and then immobilize silver wires. Afterwards, the exposed (non-implanted) ends of the silver wires were cut into suitable lengths and then burned to expose the silver layer. Unlike a metal conductor, the electrical characteristics of insects' muscle tissue are similar to a capacitor. When a pulse was applied to the muscle, a charge and discharge process happened in the muscle tissue and there is current flow in both directions.

In the tethered muscle stimulation, the electrical signals were generated from a function generator (Agilent, 33220A). The non-inserted ends of the silver wires were connected to the output of the function generator. In EMG measurements, the non-implanted ends were connected to a circuit board using alligator clips. The acquired data was transferred to the computer via a wireless communication through a base station. In freely flying electrical stimulations, a fingernail-scale wireless printed circuit board (PCB; FR4 [rigid], 500 mg) was stuck onto the pronotum of the beetle. After implanting silver wires into muscles, the wires were spread from the implantation sites to the corresponding female connectors on the board.

3.3: Tethered Experiment

3.3.1: Electrical Stimulation for Flight Initiation

In the flight initiation experiments, the muscle stimulation (DLM) and nerve stimulation (optic lobe) were conducted and compared on beetles (Figure 3.1a). For the stimulation of DLMs, two silver wires were inserted into the muscles through the scutellum to the depth of approximately 4 mm. For the stimulation of optical lobes, two silver wires were inserted near the left and right compound eyes to the depth of approximately 2 mm [76].

Figure 3.1. Anatomy of pairs of antagonistic flight muscles, the DLMs and DVMs.

(a) Overview of the dorsal side of a beetle, with the illustration of implantation sites at scutellum and head. Magnified views of dorsal thorax after (b) removal of scutellum, (c) removal of elytra, (d) exposing DLMs, and (e) exposing DVMs.

The beetle was suspended under a 20-cm-long stick, which was vertically clamped to a magnetic base (Figure 3.2). A small cubical magnet was glued to the lower tip of stick and another one was fixed on the pronotum of the beetle. With the magnets, both horizontal and vertical movements were constrained. Electrical pulse signals with amplitude 2.0 V (optic lobe) or 3.0 V (DLM), frequency 100 Hz and duty cycle 10% were applied to the optic lobe or DLM by the function generator. Even though the voltage is higher than the natural muscle potential [54, 60, 79], we observed that the stimulation voltage did not influence nearby muscles nor permanently harm the muscle tissue to destroy the normal contraction. The generated signals were monitored by an oscilloscope (Yokogawa, DL1640).

Figure 3.2. The experimental configuration of tethered flight initiation by electrical stimulation.

The stimulation effect was recorded within 5 s from the onset of signal, which was filmed at 30 frames per second. If the beetle unfolds and oscillates both wings within this period, it is counted as a success trial. The stimulations were repeated 10 times on each beetle. To avoid exhaustion of the tested beetles and to judge fairly on the success/failure of the flight initiation at every trial, even if the electrical stimulation successfully initiated flight, we stopped the flight by softly touching the wings. The rate of the number of success in the flight initiation to the number of trials is defined as the success rate. As demonstrated in Figure 3.3, the response time was determined by means of frame-by-frame playback to count the number of frames between the stimulation signal trigger (beginning of stimulation) and the first wing beat (beginning of flight). The timing of the trigger is determined by the sound marker from the function generator. The sound marker did not affect the flight initiation. No beetle reacted to the sound marker to unfold the wings (N = 5 animals, n = 100 trials).

Figure 3.3. Flight initiation was detected through visual and audio recordings. The response time is the elapsed time from the beginning of the electrical stimulation of DLM to the beginning of flight.

The stimulation was followed by the damage extent test (free flight ability test) to judge whether the electrical stimulation led to crucial damage to the beetle flight ability. After the stimulation experiment, each beetle was thrown into the air to naturally initiate flight. If the beetle can fly for longer than 10 s, it is counted as a pass in the damage extent test. Otherwise it is counted as a failure.

3.3.2: Foreleg Motion Tracking under Visual Stimulation

As demonstrated in literature review, visual stimulation (optical flow of dark and bright stripes) can induce fictive turnings in flying insects [59, 62]; thus, we chose this type of visual stimulation to determine the steering ability of beetle forelegs.

The beetle was tethered to constrain its flight within the range of a universal coupler (Figure 3.4). Thus, the beetle was capable of rolling or pitching its body, but yaw rotation was restricted. The beetle was placed ~20 cm in front of a translucent screen, which was used for projecting the wide-field optical flow patterns (dark and bright stripes) that moved leftward or rightward [35]. The stripes are 35 mm in width with a contrast rate of 2.5 Hz. In left stimulations, the stripes were moving from right to left and induced leftward turnings, and vice versa. Both left and right visual stimulations lasted 10 s, and the presentation of the two stimulations was alternated. There was a 5 s interval between the stimulations. Thus, one complete visual cycle lasted 30 s.

To track the locomotion of both forelegs, three retro-reflective markers were placed on the beetle, and a referential marker was placed at the rotation center of the coupler. As shown in Figure 3.4, one marker was placed at the posterior end of the pronotum and the other two markers were placed at the tips of both foreleg tibiae. Moreover, the distances between the foreleg coxae and pronotum marker were measured. A motion capture system (Vicon) consisting of six T40s cameras with a resolution of 4 megapixels (2336×1728) was used to detect the 3D coordinates of the markers by tracking the retro-reflective markers [97, 98]. The coordinates exported from Vicon were recorded at 150 Hz and synchronized with the visual stimulation.

Figure 3.4. The configuration of visual stimulation. In visual stimulation, a projector and a transparent screen were used to present the optical flow, and six T40s cameras were used to track leg positions. The beetle was suspended under a universal coupler, and four retro-reflective markers were placed onto the beetle. Two leg markers were placed at the tips of both tibiae, the pronotum marker was placed at the posterior end of the pronotum, and a referential marker was placed at the rotation center of the coupler.

The effects of the visual stimulations were assessed by analyzing the lateral movement of the pronotum marker. When the beetle needs to roll its body toward the turning direction, it will swing the free end of the coupler as the coupler is always perpendicular to the body. Then the free end of the coupler will be swung an angle same with roll around the center of coupler, which will move the beetle laterally to the opposite direction of turning. Thus, the visual stimulation was effective when the pronotum marker shifted to an opposite side to the direction of stimulation. All data was extracted from the period when the stimulation was effective.

The coordinate system for the calculation was based on the beetle body, which defined the heading direction as the x-axis and a plane parallel to body neutral surface as the XY-plane [99]. As body neutral surface was not always in parallel with the ground, the coordinates need to be transformed because raw data from Vicon system was based on ground coordinate system. The coordinate origins of both coordinate systems were set at a same point, the static marker. The general equation of a spatial coordinate transformation can be expressed as follows:

$$\begin{bmatrix} X' \\ Y' \\ Z' \end{bmatrix} = R_x(\varepsilon_x)R_y(\varepsilon_y)R_z(\varepsilon_z) \begin{bmatrix} X \\ Y \\ Z \end{bmatrix} + \begin{bmatrix} \Delta X \\ \Delta Y \\ \Delta Z \end{bmatrix},$$

$$R_x(\varepsilon_x) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos \varepsilon_x & \sin \varepsilon_x \\ 0 & -\sin \varepsilon_x & \cos \varepsilon_x \end{bmatrix},$$

$$R_y(\varepsilon_y) = \begin{bmatrix} \cos \varepsilon_y & 0 & -\sin \varepsilon_y \\ 0 & 1 & 0 \\ \sin \varepsilon_y & 0 & \cos \varepsilon_y \end{bmatrix},$$

$$R_z(\varepsilon_z) = \begin{bmatrix} \cos \varepsilon_z & \sin \varepsilon_z & 0 \\ -\sin \varepsilon_z & \cos \varepsilon_z & 0 \\ 0 & 0 & 1 \end{bmatrix},$$

where ε_x , ε_y and ε_z are the rotation angles around the three axes (x-axis, y-axis and z-axis) and R_x , R_y and R_z are their corresponding rotation matrixes, $[X' \ Y' \ Z']^T$, $[X \ Y \ Z]^T$ and $[\Delta X \ \Delta Y \ \Delta Z]^T$ denote the transformed coordinates, original coordinates, and translation vector of coordinate origins respectively.

As there was no translation of origin, the translation vector $[\Delta X \ \Delta Y \ \Delta Z]^T$ in the equation could be neglected. The rotation of yaw was constrained in this experiment so that angle ε_z always equaled to zero and R_z could be neglected. The angle ε_x and

angle ε_y were able to be obtained by calculating the vector pointing from the pronotum marker to the referential marker in geodetic coordinate system. Thus, the derived equation of a spatial coordinate transformation was expressed as follows:

$$\begin{bmatrix} X' \\ Y' \\ Z' \end{bmatrix} = R_x(\varepsilon_x) \cdot R_y(\varepsilon_y) \cdot \begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} \cos \varepsilon_y & 0 & -\sin \varepsilon_y \\ \sin \varepsilon_x \sin \varepsilon_y & \cos \varepsilon_x & \sin \varepsilon_x \cos \varepsilon_y \\ \cos \varepsilon_x \sin \varepsilon_y & -\sin \varepsilon_x & \cos \varepsilon_x \cos \varepsilon_y \end{bmatrix} \cdot \begin{bmatrix} X \\ Y \\ Z \end{bmatrix}.$$

Figure 3.5. The angular displacement was calculated from the angle between x-axis and leg vector, pointing from coxa to tibia, where the counterclockwise direction from x-axis was defined positive.

The angular displacement of the leg was defined as the angle between the heading direction and the leg vector pointing from the coxa to tibia on the body's coordinate system (Figure 3.5). Angular displacements that go counterclockwise from x-axis were defined positive and all of the angular displacements discussed in the study are referring to their absolute values. The swing direction of the left (right) foreleg was determined clockwise (counterclockwise) when the average angular displacement of

the stimulation decreased from the average angular displacement of the stimulation right before it, and vice versa.

3.3.3: EMG Measurement under Visual Stimulation

EMG measurement was conducted under visual stimulation. Two silver wires were implanted into the tissue of the target muscle to collect muscular potentials (unilateral EMG) during tethered flight [35]. To avoid collision with the wings, the wires were spread along the coupler. The two non-implanted ends of silver wires were connected to the input of a signal amplifier (LT1920, Linear Technology Corporation) using alligator clips. The amplified signal was transmitted to a microcontroller-based (CC2430, $7 \times 7 \text{ mm}^2$, 130 mg, 32 MHz clock, 2.4 GHz IEEE802.15.4-compliant RF transceiver, Texas Instruments) development board (SOC_BB 1.1 and CC2430EM 1.2, Chipcon AS). Using the analog to digital converter (ADC) on the microcontroller, the collected data were digitalized for wireless transmission based on the IEEE 802.15.4 protocol.

Figure 3.6. Anatomical view of the antagonistic protraction/retraction muscle groups [100]. The protraction/retraction muscle groups locate inside the prothorax connecting the coxa to the pronotum, and they control the protraction/retraction motion of forelegs.

The EMG signal of left protraction muscle was measured and analyzed in our experiment (Figure 3.6). To avoid possible interference, the right protraction muscle was also implanted with silver wires. The recorded electrical signals were processed by custom program on computer. The EMG spikes were selected based on 5-sigma control limits and synchronized with visual stimulations. Specifically, the EMG spikes that occurred during the left and right visual stimulations and intervals without stimulation were assigned to their corresponding groups to compare the occurrence rate.

3.3.4: Torque Measurement for Foreleg Swing

Torque measurements of the beetle body were collected with a torque sensor (Nano17 Titanium, ATI Industrial Automation) in tethered condition [101]. The sensor was fixed vertically downward to the ground, and a custom holder was connected right below the sensor. The holder was designed to suspend the beetle at its pronotum. The z-axis of the coordinate system was vertically upward while the x-axis and y-axis were pointing forward and leftward, respectively. As measured in the free flights with the method proposed in section 3.5.1, the pitch angle of beetle body was $28.38 \pm 3.65^\circ$ (mean $\pm$ SD; N = 5 beetles, n = 25 flights). Thus, we adjusted the pitch angle of tethered beetle to 28° . The beetle was stuck to the holder by beeswax and two thin silver wires were implanted into the left foreleg protraction muscle. The two non-implanted ends of silver wires were connected to

the output port of a function generator (33220A, Agilent) using alligator clips. Prior to stimulation, the left foreleg was forcibly spread out to its flight position.

Leg motions were elicited with the monophasic square pulses from the function generator (0.7 V amplitude, 100 Hz frequency, 10% duty cycle, and 500 ms duration). By applying the electrical signals from the function generator, the torque data were recorded with a sampling rate of 1000 Hz. The induced torque on the body, which was defined as the change of torque after stimulation, was extracted after synchronizing the torque data with electrical stimulations. Ten stimulations were conducted on each intact beetle which means that all of the legs functioned normally. Ten additional stimulations were applied after leg amputation (i.e., the left foreleg was separated from its coxa) as a control group.

Figure 3.7. The scanned 3D beetle model. The front view, left view, top view and oblique view of the model was shown in upper left, upper right, lower left and lower right, respectively.

Moreover, we scanned the legs, pronotum, abdomen and elytra of a beetle with a commercial 3D scanner (EinScan-Pro, Shining 3D; 0.05 mm accuracy). The scanned point-cloud model was imported to 3D modelling software (SolidWorks 2016, Dassault Systemes) and the model was split into parallel layers in 1 mm spacing. A digital model was built by lofting the cross sections of the layers (Figure 3.7). We measured the weight of each part of beetle and input the average weights into the digital model ($N = 5$ beetles). Then we rotated the model 28° along the pitch axis to match the real flight orientation. The moment of inertia was calculated by the software. As torque equals to the product of moment of inertia and angular acceleration, the relationship between the rotation of body, θ_{body} , and the torque induced by leg motion, T_{leg} , is as follows:

$$\theta_{\text{body}} = \int \omega_{\text{body}} = \iint \alpha_{\text{body}} = \frac{\iint T_{\text{leg}}}{I_{\text{body}}},$$

where ω_{body} , α_{body} , and I_{body} are the angular velocity, angular acceleration and moment of inertia of beetle body, respectively.

3.4: Insect-body-mountable Wireless Devices

3.4.1: Wireless IMU Backpack

The body attitudes during untethered flight were acquired by a wireless IMU backpack mounted on the insect body. Figure 3.8a shows the circuit diagram of the designed radio frequency (RF) transmission and motion sensing device. From this schematic, a custom PCB (FR4 [rigid], $15 \times 15 \text{ mm}^2$, 640 mg with components) was manufactured and soldered (Figure 3.8b). The RF transmission was implemented by a CC2530 microprocessor ($6 \times 6 \text{ mm}^2$, 32 MHz clock, 2.4-GHz

IEEE 802.15.4-compliant RF transceiver; Chipcon, Texas Instruments) and a miniature antenna (CAN4311712002451K; $3.2 \times 1.6 \text{ mm}^2$, 2.45GHz; YAGEO). The IMU backpack also contained a 9-axis integrated MPU-9250 motion sensor ($3 \times 3 \text{ mm}^2$; 3-axis gyroscope, 3-axis accelerometer, 3-axis magnetometer; InvenSense, Inc.). The CC2530 microprocessor calculated the Euler angles (the yaw, roll, and pitch angles) based on the MPU-9250 data by a customized motion processing algorithm.

The attitude angles were wirelessly transmitted to a transceiver station connected to a computer with sampling rate of 100 Hz. The received data were stored and displayed by custom designed software programmed on the computer (Figure 3.8c). The stored data were analyzed by the mathematical software MATLAB (R2012b, MathWorks Inc.). The average power consumption of the IMU board (98.5 mW) was measured with an oscilloscope (DL1640, Yokogawa). The power supply was from a rechargeable lithium ion battery (3.7 V, 350 mg, 8.5 mAh; Micro Avionics). The battery was mounted onto the top surface of the IMU backpack with double-sided tape and was connected to the power jack through the female connectors on the IMU backpack. The flight trajectory was detected by the motion capture system, Vicon. For this purpose, the surface of the battery was wrapped with retro-reflective tapes (Silver-White, Reflexite). The circuit assembly with the battery weighed approximately 1.30 g.

Figure 3.8. IMU backpack assembly and its working principle.

(a) Schematic of 9-axis inertial sensor (MPU-9250) and microprocessor (CC2530) with wireless communication functions. (b) The manufactured IMU backpack (dimensions: $15 \times 15 \text{ mm}^2$; masses: PCB assembly = 640 mg, PCB assembly + battery = 1310 mg) was photographed with a battery (upper) and without the battery from top side (lower left) and bottom side (lower right). (c) IMU backpack communicated with transceiver station connected to user's computer via wireless communication (IEEE 802.15.4).

3.4.2: Influence of Backpack Loading on Flight Performance

Before carrying out the freely flying experiments, the influence of the IMU backpack on beetle flight needed to be clarified. Three different loadings were stuck onto the beetle, which weights 0.30 g, 1.30 g and 3.60 g. The lightest loading was a slice of retro-reflective tape and the heaviest loading was a retro-reflective tape

wrapped sheet metal. The IMU backpack assembly was the 1.30 g loading. Before releasing the beetle into air, the three different loadings were respectively stuck onto the posterior pronotum with double-side tapes and beeswax (Figure 3.9). In the experiment, a flight sample contained three consecutive flight trajectories with the three different loadings, respectively. The effects of weight loading on flight performance were normally determined by the average flight speeds [102, 103]. Here the average flight speeds were determined by statistically computing all flight samples.

Figure 3.9. Photograph of backpack mounted beetle from top view (left) and side view (right). The backpack was fixed at the posterior pronotum of beetle.

3.4.3: Accuracy Test of IMU Backpack

The accuracy of the IMU backpack needs to be verified and confirmed before putting it in use on beetles. A motion capture system (Vicon), which was composed by six T40s cameras with resolution of 4 megapixels (2336×1728) and one Giganet server, was introduced to provide the required spatial coordinates. Since the Vicon system can sense and track any retro-reflective materials, a wand with four

retro-reflective markers was used to calculate the referential attitude angles (Figure 3.10a). To be specific, marker A, B and C were arranged on one straight line and marker D was placed perpendicular to the line with the foot point at marker B. Even though Vicon system was widely used to offer precision spatial coordinates [97, 98], the accuracy and consistency may be different because of the detecting space and camera configuration. By quickly moving and rotating the wand in the detection area, the angle $\angle CBD$ was computed to check the spatial consistency of our Vicon system. As the angular displacement revealed small deviation throughout the process, which was $89.93 \pm 0.06^\circ$ (mean $\pm$ SD), the angles calculated from the Vicon system can be regarded as the referential values. Then a piece of IMU backpack was fixed to the frame of the wand with the same coordinate orientation. By waving the wand for more than 6 s under Vicon system, the markers' coordinates and the data from the board were collected and recorded independently. Thereafter, the yaw, roll and pitch angles were extracted from the experimental recordings of the Vicon system and the IMU backpack, respectively. By synchronizing the results from both systems, which is represented in Figure 3.10 b-d, angles transmitted from the circuit board were compared with the ones calculated from the Vicon system. The comparison was quantitated by means of the root mean square errors (RMSE) and the Pearson's correlation coefficients.

Figure 3.10. Comparison on angular accuracy between Vicon system and IMU backpack.

(a) Four retro-reflective markers (A-D) on the wand were used to set the coordinate and calculate the Euler angles in Vicon system. The angles of yaw (b), roll (c) and pitch (d) from the IMU was synchronized with the Vicon data. The blue solid lines and black dashed lines represent the results from the IMU and the Vicon system, respectively.

3.4.4: Wireless Backpack for Electrical Stimulation

The free flying experiment was completed in a motion capture lab (dimension: 16 m $\times$ 8 m $\times$ 4 m). The lab was equipped with a motion capture system (Vicon) containing twenty T40s and T160 cameras fixed along the upper edge of the room. The custom-made computer software (BeetleBrain v.0.99b) was used to generate

signal commands via a guidance and inertial navigation assistant (GINA, provided by Professor Kris Pister's laboratory at University of California, Berkeley) base station (2.4 GHz IEEE 802.15.4 wireless protocol). The commands were received and processed by an electrical wireless backpack described by Sato, Vo Doan [35]. Specifically, the wireless stimulator backpack was developed based on Chipcon Texas Instruments CC2431 microcontrollers (2.4-GHz IEEE802.15.4 system on a chip). A custom PCB (FR4 [rigid], 500 mg) was designed and manufactured for the stimulator based on the circuit diagram. The stimulator was assembled by soldering the electronic components onto the PCB, including a microcontroller, capacitors, resistors, oscillator, terminal headers, and the antenna, as shown in Figure 3.11. Electrical signals were generated using two independent channels into the left protraction muscle and right protraction muscle, respectively. Each muscle was implanted with a pair of silver electrodes including a working electrode and a counter electrode. The muscles were unilaterally stimulated in the experiment. A rechargeable lithium ion battery (3.7 V, 350 mg, 8.5 mAh; Micro Avionics) was connected to the backpack, and the surface was wrapped with retro-reflective tape (Silver-White, Reflexite). The backpack assembly was attached to the pronotum of beetle to enable the detection of 3D flight trajectories using the motion capture system.

Figure 3.11. Photographs of the backpack (PCB + components = 690 mg, assembled backpack + battery = 1351 mg). Two channels, including two output pins for each were used as stimulating signal generators that were connected to the electrodes via the female headers [35].

3.5: Free Flight Experiment

3.5.1: Measurement of Body Attitudes on Flying Beetle

To collect the data of the three body attitudes, which are the Euler angles, the custom designed IMU backpack was stuck onto the flying beetles with double-side tapes and beeswax. After the activation of the IMU backpack and the computer software, the beetle was placed on a horizontal plane for more than 10 s to settle down the sensors and eliminate the errors by misalignment. Thereafter, the flight experiments on beetles were carried out in the motion capture lab ($16 \times 10 \times 4.5 \text{ m}^3$). A motion capture system (Vicon) with twenty T40s and T160 cameras was fixed around the upper edge of the lab. While the backpack loaded beetles were flying within this room, the ground truth system was used to record the flight trajectory. Since the system was only sensitive to the retro-reflective materials, the battery of the IMU backpack was wrapped with retro-reflective tape on the surface beforehand. Meanwhile, the IMU backpack kept sensing and transmitting data

wirelessly to the transceiver station, which was connected to a computer, with a frequency of 100 Hz (Figure 3.12). The data was collected and stored by custom software in the computer.

Figure 3.12. Time series of the attitude angles and angular velocities from IMU backpack.

(a) Attitude angles of a freely flying beetle were presented. The yaw, roll and pitch angles are drawn with red, blue and green lines, respectively. (b) Time series of the calculated angular velocities in yaw, roll and pitch were plotted with red, blue and green lines as well.

The recorded flight trajectory from the Vicon system was used to verify the accuracy of the IMU backpack. The yaw angles calculated from Vicon system were synchronized with the yaw angles extracted from IMU backpack. Once the results from both systems showed a correlation coefficient over 0.9, we concluded that the IMU backpack functioned well during the flight test and the transmitted yaw data was comparably accurate. As the yaw angles were calculated from the quaternions which were updated inside the micro-processor, the other two Euler angles, which were calculated from the quaternions as well, can be trusted. In the analysis, only

the flight segment before the collision onto the wall of the lab was picked out as the flight trajectory, which was because the collision may change the orientation of the IMU backpack with respect to the body of beetle. Moreover, the picked segment needed to last at least 2.5 s in order to make sure that the beetle still had normal flying ability throughout this trial. The moving tendencies of the angular displacements and the angular velocities, as shown in Figure 3.12a and figure 3.12b, were processed by custom MATLAB program with the purpose of analyzing body attitudes. The fluctuations, which are defined as the signals over 3 Hz in frequency [104], on roll angles and yaw angular velocities were extracted by using a high-pass Chebyshev filter (cut-off frequency of 3 Hz). Then roll angles and yaw angular velocities, as well as their fluctuations, were synchronized on the same timeline. The flight turnings were the unidirectional segments in flight trajectories whose maximum amplitudes in roll angle were over 20° and durations were over 0.35 s. Thus, one trajectory may contain more than one turning. The maximum yaw velocity and the maximum roll angle of each turning were picked out for summarizing the relationship on amplitudes between roll angle and yaw velocity. The roll angles were approximately separated into 18 intervals from -45° to 45° with a step length of 5° and the yaw velocities were grouped to the intervals by their corresponding roll angles. Then average yaw velocity of each interval was calculated to observe the relationship between roll angles and yaw velocities. Moreover, Pearson's correlation coefficient was chosen to quantify the correlation between roll angles and yaw velocities, both of which were filtered to smooth the lines. Moreover, the coefficient was calculated under different time lags, which was defined as the time difference between yaw and roll from -200 ms to 200 ms with a

step length of 20 ms, for the sake of comparing the timing sequence. For each flight trajectory, the above calculations were carried out and only by statistically counting all samples, the results and conclusions were discussed.

3.5.2: Electrical Stimulation on Foreleg Muscle in Flight

For observing the effects of foreleg movements on flight course, electrical stimulations were applied on foreleg muscles during free flight. This experiment was carried out in the motion capture lab with the Vicon motion capture system which contained twenty T40s and T160 cameras (Vicon, Oxford, United Kingdom) fixed along the upper edge of the lab (Figure 3.13a). The stimulation commands were transmitted from custom-made computer software, BeetleCommander v.1.8e, via a computer-driven wireless base station based on IEEE 802.15.4 protocol (Figure 3.13c). The commands were received and executed on a custom wireless backpack which was mounted on the pronotum of a flying beetle (Figure 3.13b). The backpack could modulate pulse signals into different frequencies, voltages and durations according to the received commands. Pulse signals could be generated on two independent channels, the left channel and the right channel. The left channel was connected to the protraction muscle of left leg. Likewise, the right channel was connected to the protraction muscle of right leg. The electrical stimulation of the left or right protraction muscle induced the swing of the corresponding leg clockwise or counterclockwise (viewed from the dorsal side of the beetle). Moreover, a Nintendo Wii remote was used as a manual remote to trigger the left or right channel by communicating with BeetleCommander v.1.8e over Bluetooth communication protocol (Figure 3.13d).

Figure 3.13. The configuration of foreleg stimulation in free flight.

(a) The experiment was conducted in a flight arena of $12 \times 8 \times 4 \text{ m}^3$ equipped with a motion capture system of 20 near-infrared cameras (Vicon, T40s and T160). (b) The backpack was assembled and mounted onto the beetle before releasing the beetle into the air for free flight. The backpack received wireless signals from a laptop (c) equipped with a base station when the operator pressed the command button of the Wii remote (d). On command, the backpack applied an electrical stimulus to the implanted muscle. The coordinates of the flying beetle were recorded with timestamps by the motion capture system. These coordinates were sent to the laptop for synchronization with the stimulation command.

A preliminary experiment was conducted to determine the appropriate stimulation voltage for the protraction muscle. The function generator was used as the signal

source, which generated pulse signals with a 100 Hz frequency, 10% duty cycle, and 500 ms duration. The voltages ranged from 0.5 to 1.5 V with a step width of 0.1 V. While stimulating the foreleg protraction muscles, the EMG of flight muscles was recorded. Five flight muscles were measured, including the DLM, DVM, BSM, SBM, and 3Ax muscle. The minimum voltage that elicited regular EMG spikes on any flight muscle was defined as the threshold. The overall threshold voltage was 0.90 ± 0.07 V (mean $\pm$ SD; N = 5 beetles, n = 5 thresholds). Accordingly, the electrical stimulation was set to 0.7 V as it did not trigger the flight muscles (Figure 3.14).

Figure 3.14. EMG of flight muscles during electrical leg stimulation. The stimulation consisted of 0.7 V pulses for 500 ms (black bars). The electrical stimulation of leg muscle caused no clear EMG spikes on the flight muscles (N = 5 beetles, n = 50

stimulations), which suggests that the electrical leg stimulation does not influence flight muscles.

Prior to the free flight stimulation, induced leg motion was verified on tethered beetle. Next, the beetle was released into the air, and stimulation commands were transmitted wirelessly. The monophasic square pulse signals were set to 0.7 V, 100 Hz, and 10% duty cycle with 500 ms duration. The induced yaw torque by leg motion was evaluated by the flight turning rates [43, 105]. Based on the recorded flight trajectory, the turning rate ω can be calculated from three consecutively positions (P_1 , P_2 and P_3) on the horizontal plane as follows:

$$\omega = \cos^{-1} \left(\frac{\overrightarrow{P_1 P_2} \cdot \overrightarrow{P_2 P_3}}{|\overrightarrow{P_1 P_2}| |\overrightarrow{P_2 P_3}|} \right) / \left(\frac{t_{P_3} - t_{P_1}}{2} \right).$$

By synchronizing the flight trajectory with the stimulation commands, the induced turning rate ω_{induced} , given by $\omega_{\text{induced}} = \omega_{150} - \omega_0$, where ω_0 and ω_{150} are the turning rates immediately before stimulation and 150 ms after stimulation, respectively, was computed to analyze the stimulation effect. This experiment was completed using intact and amputated beetles. After testing each intact beetle, both forelegs were cut off from the coxae. The above electrical stimulations were repeated on the amputated beetles with the same configuration. The induced turning rates computed from the amputated beetles were compared with the results of intact beetles. For the calculation of induced turning rates, all results greater than 30° s^{-1} were excluded from the dataset because it is apparently larger than the possible effect generated by foreleg.

Chapter 4 : The Flight Initiation Induced by Electrical Stimulation

4.1: Introduction

The development of reliable MAV has challenged researchers for decades and remains actively studied today. MAVs fly and navigate into restricted and complicated spaces with flexibility and splendid controllability. Therefore, MAVs that are practically usable in real life, especially in search-and-rescue operations and indoor surveillance [106], have been a long-term ambition of researchers. As micro system technologies advance, achieving this ambition has become increasingly realistic [10, 107, 108], and researchers have developed MAVs that are smaller and more controllable. However, even state-of-the-art MAVs cannot be used over long durations with complex maneuverability because of the limited energy capacity of the power source, high power consumption rate, and complicate control systems adopted for maintaining and stabilizing the posture in air [10].

Meanwhile, insect flight mechanisms and their aerodynamic characteristics have attracted considerable interest [1, 15, 84]. The efficient motors (flight muscles) of insects enable wing flapping over a long duration and subtle alterations in the wing beat trajectory, ensuring high maneuverability in air [3]. This raises the following question: could a live insect be adopted as an MAV platform; that is, could we mount or implant a tiny electrical stimulator on a live insect, thus controlling its motor actions by stimulating its neuromuscular sites? Such insect-machine hybrids, or cyborg insects, have been actively researched [35, 65, 76, 77, 79, 80, 109-115]. Various methods have been proved effective for controlling insects, such as electrical [35, 65, 76, 77, 109, 111, 115], photic [112], and thermal stimulation [80] as well as chemical injection [79]. By combining artificial devices with live insects, we can exploit the intrinsic excellent flight performance of insects to serve human

needs. For example, the insect–machine hybrid flying robots can potentially monitor narrow and hazardous environments that are inaccessible to humans.

The first and most essential challenge of developing an insect–machine hybrid flying robot is establishing a stable flight initiation protocol for the insects. A flight initiation protocol is a requisite of a fully controlled air vehicle. Such a protocol should be highly reliable, rapidly responsive, and minimally destructive. Several methods of flight initiation have been proposed for various insects, each with its advantages and disadvantages. Specifically, cockroach flight has been chemically stimulated by octopamine and wind puff [79]. Moth flight has been successfully initiated by electrical stimulation of the brain and thorax [77]. Other researchers have electrically stimulated the optic lobes of beetle heads to initiate flight [65, 76]. Beetle flight has also been accomplished by micro-thermal stimulation at the base of the antenna [80]. Among these methods, electrical stimulation appears to be the most suitable in practice, because it is easily applied and delivers highly reliable results. However, as electrical stimulations to the head area require accurate microsurgery skills and may permanently damage the insect body, electrical stimulations at parts other than the head, the thorax for instance, should be the next focus of flight initiation. Electrodes cannot be precisely implanted and fixed in neuronal tissue, because the tiny, densely arrayed neurons are difficult to separate. In contrast, muscles are much larger and easily identified under a conventional optical microscope or even by the naked eye. We thus selected muscle as the target of electrical stimulation to induce our desired motor action, flight initiation. The primary outcome from this study is damage-less flight initiation with high success rate (> 90%). The beetle species *Mecynorrhina torquata* (order: Coleopteran) was

chosen for the study as it has a relatively large body size and a high load capacity (in flight, it can carry 20–30% of its body weight) [13, 76]. Thus, this species is a suitable platform for making cyborg insect. We investigated the indirect flight muscles, namely, the dorsal longitudinal muscles (DLMs) and the dorso-ventral muscles (DVMs), which generate the wing oscillations [1, 4]. To initiate the flight, we attempted to stimulate either of these muscles with electrical pulses.

4.2: The Selection of Target Muscle

Beetles and many other insect orders maintain wing oscillations by alternately contracting their DVM and DLM, which constitute an antagonistic pair of flight muscles. To initiate wing oscillations, either or both the DVM and DLM should be stimulated. The DVM and DLM are located in a side domain and mid-domain, respectively, in the thorax of a beetle (Figure 3.1). To implant electrodes into DVM, the elytra need to be cut and removed to expose the cuticle enclosing the DVM (Figure 3.1). We note that the elytra play a critical role in flight steering. The elytra of other Coleopteran generate lift during flight [116, 117] and the elytra form part of the mechanism that folds the hind wings [118, 119].

In fact, the removal of the elytra resulted in loss of steering. Two days following the removal of their elytra, 4 out of 5 beetles lost their flight ability within 10 s; that is, 80% of the tested beetles demonstrated significantly impaired flight ability. We also note that, since the DVM is inserted in the cuticle, part of that cuticle will be destroyed by the electrode implantation, reducing the power output of the DVM. Eventually, we concluded the DVM is not an appropriate target for the electrical stimulation.

Another option for flight initiation is stimulation of DLM, the counterpart of the DLM–DVM antagonistic pair for wing oscillation. The DLM locates underneath the thin cuticle (Figure 3.1), which is found underneath the thick, triangular-shaped cuticle referred as to scutellum. Notably, unlike beetles with elytra removed, all the beetles with scutellum removed stably flew for more than 10 s even two days after the removal ($N = 5$ animals). The removal of the scutellum does not significantly affect the free flight ability. In addition, the DLM fibers are oriented parallel to the plane of the thin cuticle and the scutellum (the DLM is inserted into the internal cuticle plate perpendicular to the thin cuticle and the scutellum). Thus, implantation of the electrodes into the thin cuticle would not significantly reduce the power output of the DLM and would not result in the loss of flight ability. In fact, when electrodes were implanted into the DLM through holes pierced in the thin cuticle, the tested beetles exhibited no obvious irregular behavior during flight. All the beetles with electrode implanted into the DLM passed the free flight ability test ($N = 5$ animals, $n = 25$ trials). Thus, the DLM should be a better choice for stimulation than the DVM.

4.3: Comparison between Muscle Stimulation and Optic Lobe Stimulation

As the optic lobes constitute the massive neural cluster of the compound eye, the electrical stimulation would likely destabilize the beetle's flight. Following Sato, Berry [76], we implanted the stimulation electrodes into the left and right optic lobes and applied electrical stimulation (2 V, 100 Hz, $N = 5$ animals, $n = 50$ trials). Same as reported in [76], the beetles unfolded and oscillated their wings reliably. In the muscle stimulation, the DLM-stimulated beetles frequently unfolded and oscillated their wings ($N = 9$ animals, $n = 90$ trials). Specifically, the DLM

stimulation successfully initiated flight in 82 out of the 90 trials, which revealed an average success rate as high as 91%.

Usually beetles thrown into the air can initiate flights spontaneously (typically, they unfold their wings, begin wing oscillation, and fly for more than 10 s). However, in the free flight ability test, all the beetles lost steerage in the air and could not sustain flight for 10 s after optic lobe stimulation ($N = 5$ animals, $n = 25$ trials). The same reaction (unfolding, oscillating but losing flight control) was found in beetles that were blindfolded by sealing their compound eyes with beeswax and plasticine ($N = 5$ animals, $n = 25$ trials). When the blindfold was removed, all the beetles recovered the flight ability and flew more than 10 s in the free flight ability test. We conclude that the electrical stimulation causes flight disturbance by crucially damaging the optic lobe. As optical lobes are the upstream of the neural network system in insects [120], their damage will disrupt the muscles downstream at the neural network terminal. Thus, we conducted the same free flight ability test after stimulating the flight muscles to verify whether muscle stimulation crucially damaged the flight ability. Notably, in contrast to the optic lobe stimulation, none of the tested beetles lost steerage in air, confirming that DLM stimulation imparted no crucial damage to the insect's flight system.

4.4: Habituation and Power Consumption for Muscle Stimulation

The DLM stimulated beetles showed little habituation. We measured the response time to the DLM electrical stimulation, defined as the time interval between the beginning of the stimulation and the timing of wing unfolding (the beginning of wing oscillation), as illustrated in Figure 3.3. Significant habituation would

manifest as lengthening response time; that is, the response time would increase as the stimulation was repeated. As shown in Figure 4.1, the average response times to the DLM stimulation in the first and second 5 trials differed by less than 0.33 s. Among all tested beetles and all trials, the response time varied by less than 23%, which means that the beetle did not significantly become habituated to the DLM stimulation. The average response time was below 1.0 s, sufficiently short for practical application; specifically, this finding is suitable for use as an insect-machine hybrid flying robot.

Figure 4.1. The response time of muscle stimulation for flight initiation. For each tested beetle, the (a) left, (b) middle, and (c) right columns indicate the average response times of the first 5 trials, all 10 trials, and the last 5 trials, respectively. The bar in each graph indicates the standard deviation.

The power consumption of the DLM stimulation was extremely low, which was in the order of 10 mW. Figure 4.2b shows a typical time course of the current flow through the DLM after stimulation. According to the recorded current and the input voltage, the average power consumption of the muscle stimulation was calculated as

11.3 mW ($N = 5$ animals, $n = 25$ trials). In contrast, the power consumption of state-of-the-art man-made miniature robots is still in the order of 100–1000 mW [121], which is over ten times larger.

Figure 4.2. (a) The pulse trains applied as electrical stimulation to DLM and (b) the current flow induced by the signal inside muscle.

4.5: Discussions and Conclusions

Many researchers have attempted to create living machine, or cyborg insect, which is a fusion of living animal and man-made devices to control locomotion of the animal. A big challenge in flight is to reliably induce the initiation of flight. However, the earlier reported methods are either too harmful for animal itself or not reliable enough for practical usage.

A new effective and reliable method for inducing the initiation of flight (wing oscillation) on a live beetle by electrically stimulating a flight muscle is required as the previously proposed nerve stimulation (optic lobe stimulation) apparently damaged the flight ability of beetle. As we know, the wing oscillations of beetles and many other insect orders are generated by alternately contracting their DVM and DLM, which constitute an antagonistic pair of flight muscles [5, 25, 64]. Thus, the DLM and DVM are studied as the potential stimulation targets. In practical operation, the DVM is hard to stimulate as it is covered by the elytra and the elytra cannot be removed as they are crucial in flight control [116, 117]. Thus, the electrical muscle stimulation was applied onto the DLM.

The result of DLM stimulation revealed a high success rate ($> 90\%$), rapid response time (< 1.0 s) with small variation (< 0.33 s). The variation in response time also indicated the little habituation. Notably, the flight muscle stimulation caused no crucial influence on the beetles' flight performance and the beetle could still steer the flight as regularly as before the stimulation whereas the earlier demonstrated optic lobe stimulation leads to the clear loss of steerage in flight. Thus, we conclude that the electrical stimulation of dorsal longitudinal muscle (DLM) causes no obvious damage in the flight control system.

In conclusion, we successfully initiated a beetle's wing beat by simple stimulation steps with low power consumption. The success rate was considerably high and the damage due to the electrical stimulation is negligible for free flight ability. Beetle responded to the stimulation quickly without clear habituation, which makes it a reliable method for practical use. Finally, we note that the wing-beat principles and muscle configurations of many insects are quite similar (namely, the down- and up-

stroke of the wing is driven by DLM and DVM, respectively) [1, 4]. Therefore, our approach should significantly contribute to the future design of insect-machine hybrid flying robots.

Chapter 5 : The Banked Turn Measured by IMU Backpack

5.1: Introduction

The study on insect flight is of close attention and intense exploration as insects may inspire the development of MAVs, especially for FWMAVs. As insects are born talent flyers in nature, they have unmatched advantages in maneuverability and stability of flight dynamics even under fast body saccades [43, 105, 122]. Meanwhile, even though artificial MAVs have been practically used in real life, the limited flexibility and controllability in flying have long been obstructions and challenges for their further applications, especially in restricted and complicated spaces [77]. Thus, studies on insect flight mechanisms and principles have attracted considerable interest because they are considered as a feasible way towards making MAVs more flexible and more stable [1, 84]. Revealing the methodologies, mechanisms and principles in flight control of insects will benefit the design of MAVs by mimicking or referring them.

Insect flight has long been studied with diverse focuses. The wings, as well as their articulations and flight muscles, have been largely studied in terms of wing kinematics, mechanics and muscular physiology and aerodynamics [23, 35, 92, 123-125]. Apart from the wings and flight muscles, the adjustment of the body in flight is also of great interest because the body posture in insect flight has been known as an essential factor [49, 50, 52, 59, 60, 65]. As reported on many insect species, the manipulation of the body streamline can alter the flight path or adjust the flying speed [52, 59, 60]. The abdomen and hindlegs are effective in assisting flight turning and compensating flight fluctuation on locusts [52, 60]. For the honeybees [59] and bumblebees [104], the abdomen is used to adjust flight directions and pitch angles. Even the rotation of head was proved visually coordinating the attitudes on locusts [49, 50]. In engineering, insect-scale flapping wing MAVs have been actually demonstrated, which partially

mimicked the insect flight [10, 107]. However, even the state-of-the-art insect-scale flapping wing MAVs are far from the real insect in flight maneuver, one limitation of which is the lack of precision attitude control [107]. Thus, studying insect attitudes is necessary to improve the MAVs.

The flight attitudes of insect body have been studied by many researchers and different approaches have been proposed towards tracking the three Euler angles. High speed cameras have been widely employed in the researches of banked turns on fruit flies and hawkmoths [43, 86, 122, 126, 127]. Tethered experiments on locusts were using the force balance to directly reveal the generated forces or torques [49, 101]. Other tethered experiments were conducted on beetles with a gimbal being attached onto, which exhibited the pitch angles [76]. Moreover, a study on wasps implemented brushless motors to intentionally adjust body roll angles [88]. However, the high speed cameras can only be focused to a limited range which means the insect cannot be allowed to freely fly in a large space. The insects may show different flight patterns in velocity and turning rate subject to the limited field. In the tethered experiments, animals cannot fully control their flight attitudes and they may exhibit wing kinematics and body postures different from their natural behaviors [92]. Thus, another approach, mounting a tiny attitude measurement unit onto the insect, becomes a concerned topic, which is inspired by the successful demonstrations on insect-body-mountable devices. In the demonstrations, two types of electrical stimulators, which were respectively designed by Bozkurt, Gilmour Jr [77] and Hinterwirth, Medina [15], were used to control the flight on hawkmoth. Moreover, a wireless potential meter was designed for being mounted on locusts to record muscular potentials [93] and the beetles were loaded with a miniature circuit board to apply electrical signals to the flight muscles [35].

Even though the IMUs have already been applied in the studies of birds [128-130], they have not been used on the insects yet because of the limited payload. With the development of micro-electronics, the appearance of tiny chips made it possible to design an insect-body-mountable IMU device, or ‘backpack’. As the first attempt in collecting insect attitudes with IMU, we need to make sure that the device accurately measures the Euler angles, wirelessly transmits the data within a large space, and does not cause any apparent interference on the insect flight. A beetle *Mecynorrhina torquata* (order: Coleopteran) was chosen as the tested animal in this study as this animal has a great payload capacity up to 30% of its own body mass or ~3.0 gram [76]. With a qualified mountable device, we attempted to measure body attitudes, which are Euler angles, on freely flying beetles. The banked turn in beetle flight was studied with the attitude angles. A main characteristic of banked turn is the coupling of roll angle and yaw velocity [131]. Thus, as has not been statistically and mathematically analyzed, the relationship between roll angles and yaw angular velocities in the banked turns was the focus in this study.

5.2: The Feasibility of IMU Backpack

In the designed schematic (Figure 3.8a), both the sensor and the micro-processor were selected based on a balanced consideration of size and accuracy. As shown in Figure 3.8b, the IMU backpack was $\sim 2.3 \text{ cm}^2$ in area and the total mass with the battery was $\sim 1.30 \text{ g}$. As proved from the testing of flight performance, beetles can maintain their flights with the backpack loaded (Figure 5.1). The flight samples ($N = 4$ animals, $n = 16$ flight samples) showed that an average flying speed of $5.56 \pm 0.86 \text{ m s}^{-1}$ (mean $\pm$ SD) was recorded when the beetles were carrying a 0.30 g retro-reflective marker. The flights with the IMU backpack and the excess load had the average flying speed of 5.57

$\pm 0.88 \text{ m s}^{-1}$ and $3.41 \pm 1.33 \text{ m s}^{-1}$, respectively. The one-way ANOVA result shows that the different loadings caused significant influence on flight speed ($p < 0.0001$) whereas the subsequent t-test proves the speeds under 0.30 g and 1.30 g loadings are statistically equal ($p > 0.995$). It means that the 1.30 g backpack doesn't influence the beetle's flight and the 3.10 g excess load will apparently influence the flight.

Figure 5.1. The flight speeds under different loadings. The flight speeds of beetle were compared under three loadings: a small marker (0.30 g), IMU backpack (1.30 g) and excess load (3.60 g). The boxplots show the median values (solid horizontal lines), mean values (white diamonds), upper and lower quartiles (box outlines), and maximum and minimum values (whiskers).

The accuracy of the IMU backpack was tested by a ground truth system (Vicon). Figure 3.10 b-d shows the yaw, roll and pitch angles which were collected from the IMU backpack and the Vicon system for comparison. To quantify the deviation between the two data sources, the Pearson's correlation coefficients and the root mean square errors (RMSE) were computed for the three Euler angles ($n = 8$ trials). The correlation coefficients of yaw, roll and pitch with respect to the Vicon results were 0.983 ± 0.009 ,

0.985 ± 0.009 and 0.979 ± 0.018 , respectively (mean $\pm$ SD). The 99% confidence interval (CI) of mean was carried out to support the correlation. The results showed that the lower limit of the CIs of the three angles were all larger than 0.95. The RMSE values found on yaw, roll and pitch axes were in the values of $1.15 \pm 0.30^\circ$, $2.28 \pm 0.66^\circ$ and $1.10 \pm 0.26^\circ$, respectively (mean $\pm$ SD). As a comparison, the fluctuations on roll axis (Figure 5.2a), which is the smallest roll maneuver discussed in this study, had an average angular amplitude of $11.20 \pm 4.05^\circ$ (mean $\pm$ SD; $N = 10$ animals, $n = 69$ fluctuations), apparently larger than the maximum RMSE.

Figure 5.2. The analysis on the correlation between roll angles and yaw angular velocities.

(a) Representative time series of the roll angles (blue solid line) was synchronized with the corresponding yaw angular velocities (red solid line). By using a high-pass filter (cut-off frequency of 3 Hz), the fluctuations of the roll angles (blue dashed line) and the

yaw velocities (red dashed line) were extracted. **(b)** The mean correlation coefficients between roll angle and yaw angular velocity (black dotted line) with SD (grey shaded area) were calculated under different time lags ($N = 10$ animals, $n = 69$ trajectories). Positive lag represents the timing of the yaw angular velocity is shifted earlier than the roll angle. **(c)** Maximum yaw velocity and maximum roll angle in a turning was picked out and plotted. The points aggregated along a straight line ($N = 10$ animals, $n = 140$ turnings). **(d)** Average yaw angular velocities (black dotted line) with SD (grey shaded area) under different roll angles were linearly distributed ($N = 10$ animals, $n = 69$ trajectories). The roll angle was approximated to a nearby integer which was predetermined with an interval of 5° .

5.3: The Correlation between Yaw Velocity and Roll Angle

The curves of the roll angles and the yaw velocities were positively correlated to each other. To quantify the correlation and compare the timing sequence between roll angles and yaw velocities, the Pearson's correlation coefficient was computed under different time lags (Figure 5.2a). By statistically processing all flight trajectories in Figure 5.2b, the highest correlation coefficient is found over 0.91 ($N = 10$ animals, $n = 69$ trajectories). The high coefficient confirms the correlation between the roll angles and the yaw velocities. Moreover, the result also supports that the highest coefficient occurs at the timing of -60 ms, which means that the change of roll angle happens ~60 ms earlier than the change of yaw angular velocity in a turning. By individually counting the timings of highest correlation on all trajectories, the advanced time of the roll angle was found 58 ± 57 ms (mean $\pm$ SD). A two-tailed t-test ($p < 0.001$) significantly confirmed the timing difference between the roll and the yaw.

5.4: Maximum and Average Yaw Velocities under Different Roll Angles

As to the maximum yaw velocity versus maximum roll angle of the flight turnings (Figure 5.2c), the data points distributed along a dashed line ($N = 10$ animals, $n = 140$ turnings), which is $Y = 4.79X + 17.67$, $R^2 = 0.66$ according to the least square linear regression estimation. It means that the maximum body banking was in proportion with the maximum turning rate in heading direction. The above statistics focused on the amplitudes of roll angle and yaw velocity and did not restrict the timing sequence of maximum roll angle and maximum yaw velocity, which means the maximum yaw velocity could happen not on same time with the maximum roll angle. Thus, another analysis was conducted by synchronizing each roll angle to its corresponding yaw angular velocity, by which the average values were computed rather than the maximum values. By setting up roll intervals, the average yaw velocities versus roll angles demonstrated that the roll angles, which were from -45° to 45° , roughly kept a linear correlation with the averaged yaw velocities (Figure 5.2d). The equation of least square linear regression is $Y = 3.61X - 4.64$, $R^2 = 0.98$.

5.5: Discussions and Conclusions

The designed IMU backpack is applicable for studying the free flight of beetles owing to its miniature size. The area of the IMU backpack is even smaller than the pronotum of beetle and the mass equals to $\sim 17\%$ of that of a beetle. As proved from the testing of flight performance, the flight speed with the IMU backpack mounted was comparably equal to the speed without the IMU backpack whereas the speed was significantly reduced after attaching the excess load. Since Srygley and Kingsolver [103] analyzed the average flight speed and concluded that insects can adapt to additional weight

through manipulating the attack angles of wings, the negligible difference in flight speed proved the beetles overcame the weight of the IMU backpack and maintained their regular flight performances. Thus, the IMU backpack can be mounted onto the flying beetles without influencing their natural flight performance.

The accuracy of the data acquired by the IMU backpack is acceptable for this research. The largest RMSE of the IMU backpack was found in the value of 2.28° and the correlation coefficients on three axes were all around 0.98. These results are comparably close to previously reported attitude and heading reference systems (AHRS) which implemented inertial sensors with similar accuracies and resolutions [97, 98, 132-134]. In these studies, the data from their IMU devices were also compared with the ones from reference systems, such as Vicon [97, 98] or commercial inertial navigation system (INS) [133]. To be specific, the RMSE results were found between 1° and 3° on the reported navigation platforms [132, 133]. As mentioned in the other researches on correlation coefficient [98, 134], the results between different navigation systems varied from 0.95 to 0.99. Moreover, as the smallest roll maneuver discussed in this study, the roll fluctuation, has average angular amplitude ($11.20 \pm 4.05^{\circ}$) nearly 5 times larger than the RMSE, the relatively small error caused by the IMU backpack cannot qualitative influence the analysis on the attitudes. Thus, the design of the IMU backpack is accurate enough for this study.

From the free flight experiment using the IMU backpack mounted beetles, we found the roll angles were changing along with the yaw angles in flight turnings (Figure 3.12a). Similarly, aircrafts perform banked turns by simultaneously rolling their bodies until the aerodynamic force vector tilts into the desired direction [86, 105, 131]. By mathematically analyzing the banked turns on fruit flies, Cheng and Deng [131] proved

the roles of the in-phase coupling of roll and yaw in flight stabilization. Thus, it is known that the roll angle plays a significant role in the directional control. This study aimed at the relationship between the roll angle and the yaw angular velocity, which is quantified by computing the correlation coefficient, maximum yaw angular velocities and average yaw angular velocities.

The coupled controlling method on roll angle and yaw angular velocity is discussed and verified. The high coefficient, which is over 0.91, confirms the strong correlation between the roll angles and the yaw velocities. The correlation on the two variables should be the result of the disequilibrium of the forces generated from the wings for inducing a flight turning. In the turning, flapping insect generates a pair of disequilibrium forces by the wings to produce a yaw torque. As the forces generated by the wings have an ascending angle from the horizontal plane [43, 62], the disequilibrium forces of the wings also changes their vertical component forces concomitantly, which generates a torque about the roll axis. This is the coupled effect between the roll angles and the yaw velocities, which explains the highly correlated phenomenon.

To study the extent of the coupled effect, the rules of the correlation behind the roll angles and the yaw velocities need to be analyzed. Since roll angle and yaw velocity are highly coupled, the certain quantitative correlation should be found. The maximum yaw velocity in a turning roughly follows a linear relationship with respect to the maximum roll angle. Thus, a larger maximum roll angle in a flight turning will appear under a faster rotation in yaw. However, the above statistics focus on the amplitudes of roll angle and yaw velocity and do not restrict the timing sequence of maximum roll angle and maximum yaw velocity, which means the maximum yaw velocity may happen not

on same time with the maximum roll angle. Thus, the analysis on average yaw angular velocities is conducted. The result mathematically proves that the roll angles keep a linear relationship with the average yaw velocities. Both analyses above lead to the conclusion that the coupled relationship between roll angle and yaw angular velocity is linear on flying beetles. Moreover, the strong correlation and the linear relationship also reveal the other controlling mechanisms on roll, if exist, cannot qualitatively influence the coupled effect during a directional control. So it is believed that the coupled effect generated by the wings is dominant in the roll manipulation during a banked turn.

The timing difference between roll and yaw velocity should be attributed to the non-coupled roll control. The result of correlation coefficient supports that the change of roll angle happens ~60 ms earlier than the change of yaw angular velocity in a turning. Similar to our finding, Beatus, Guckenheimer [92] reported that the roll change happened earlier than yaw in flight perturbations in fruit fly. This phenomenon should not happen under the simplex coupled effect because, as mentioned above, the roll change is a by-product of the turning in yaw in the coupled correlation and it cannot appear earlier than the yaw change. Thus, the roll rotation happened with no coupling with yaw angular velocity before the yaw rotation. Wagner [126] proposed a kind of non-coupled roll control by proving that the independent roll manipulation with respect to yaw must exist on a flier which does not produce a side-thrust. Thus, even though the non-coupled roll control is far not as obvious as the coupled effect, it still can be believed that the non-coupled roll control should be responsible for the timing difference between roll and yaw velocity.

The evidence of the active roll manipulation is found in the recording of roll angular velocities. Apart from the passive aerodynamic damping, which always exists in the

flight manipulations [86, 92, 105], the existence of active control on the roll rotation needs to be clarified. Muijres, Elzinga [86] discussed that the passive damping effect in roll control only asymptotically reduces roll velocities but not reverse them whereas the active production of counter-torques can reverse the roll velocities. For example, when a roll angular velocity appears, as seen at the beginning stage of a fluctuation, the passive damping effect reduces the angular velocity until it becomes zero while the active control has the ability to produce a counter-torque to reverse the direction of the angular velocity. As a bit frequent (4.32 ± 0.63 Hz; $N = 10$ animals, $n = 69$ trajectories) fluctuations of roll were found on all recorded trajectories, these fluctuations could be found on the calculated roll angular velocities as a consequence. As shown in Figure 3.12b, the directions of roll angular velocities were apparently and consecutively reversed in the fluctuations. Consist with the above analysis of Muijres, Elzinga [86], the existence of active adjustment on roll should exist in beetle flight. However, we cannot analyze the extent of the active control in insect flight with the absence of detailed kinematics, aerodynamics, and flight models. Thus, the finding implies that the active roll manoeuver should be a practical method in flight control as well.

In conclusion, a custom IMU integrated insect-body-mountable backpack was demonstrated to measure body attitudes on flying beetles. The IMU was accurate sufficiently to track the angular maneuver of freely flying beetles. The low weight and small size of the IMU backpack allows for the remote radio recording without bothering the flight. Through the free flight experiment, we found a strong coupled correlation between the roll angles and yaw angular velocities. The calculations on correlation coefficients proved the existence of coupled effect between the two variables. Moreover, a linear correlation was quantified by computing the average yaw velocities. Apart from

the strong correlation, the delay of the change in yaw angular velocity from that in roll angle should be attributed to the non-coupled roll control. Moreover, the fluctuations in roll angular velocity suggest that the roll can be adjusted actively as well. The IMU backpack used in this study should be helpful to explore motor actions and behaviors of small animals even in air.

Chapter 6 : The Function of Forelegs in Flight Control

6.1: Introduction

Insects reveal unmatched flying abilities even in the unsteady air flows [46, 77, 92]. Multiple body parts other than the wings, such as abdomen [60-62, 84, 90], head [49, 50], and legs [51, 54, 55, 57], are found effective in controlling flight direction or adjusting flying velocity. The insects' precise and efficient flights cannot be achieved without the collaboration of different mechanisms all over the body [52, 53, 56, 101, 105, 126, 135, 136]. Many researchers have long been striving to uncover the roles of different body structures in flight manipulation. In their studies, various topics have been paid attention to, for example, the postural control [85, 137, 138]. In our research, the beetle, *Mecynorrhina torquata*, is used to investigate the roles of the forelegs in maintaining the flight.

In the descriptions of insects' flying postures, the forelegs are often mentioned as forelegs are folded or pressed closely against the body [75, 139, 140]. This is commonly seen on the insects such as butterflies, locusts, dragonflies, moths, and bees. Different from most insects, beetles fully lift and outstretch their forelegs during flight (Figure 6.1). Moreover, many beetle species have longer and thicker forelegs than the midlegs and hindlegs, which are also different from most flying insects [71]. This uncommon behavior seems to be unadvisable because it will apparently increase the air drag [57]. Meanwhile, beetles often swing their forelegs while in flight. According to the physical principle of conservation of angular momentum, when a leg rotates (swings) about the leg base (coxa), the body of the flying beetle should rotate in the opposite direction [58]. A long and relatively heavy foreleg will have a relatively large moment of inertia so that the resulting torque exerted on the body might be large enough to significantly rotate the body in flight. Accordingly, the idea that the outstretching of forelegs may be

intentionally elicited in order to help control the flight comes to our mind. If so, it is necessary to uncover the reason why forelegs are lifted and stretched in flight.

Figure 6.1. Inflight postures of the beetle *Mecynorrhina torquata* (top left), butterfly *Leptidea amurensis* (top right), dragonfly *Anax parthenope* (bottom left), and honey bee *Apis mellifera* (bottom right). The flying beetles always outstretch their forelegs [71], whereas other insects tend to fold or press their forelegs closely against the body during flight [75, 139, 140]. The photos except on the top left are courtesy of photographer Mr. Kazuo Unno. The photos are used with permission.

It is undeniable that the wings, as well as their articulations and flight muscles, are the dominant mechanism in controlling insect flights [1, 5]. Researchers have revealed their functions in terms of wing kinematics [99], muscular physiology [93] and aerodynamics [43]. However, as reported on many insect species, the auxiliary flight controls from the

body structures apart from wings are also known as essential factors. To be specific, both the abdomen and hindlegs are effective in assisting flight turnings and compensating flight fluctuations by swaying them laterally on locusts [51, 52, 61, 136]. Similarly, the abdomen and hindlegs of flies take important roles in flight control [56, 62]. The honeybee widely manipulates its body streamline to adjust the flying speeds and the heading directions [59, 140]. Meanwhile, the bees make use of the hindlegs to stabilize the bodies in flight [57, 58]. It is also found the elytra of beetle are placed asymmetrically in flight turnings [65]. Even the rotation of head is found visually coordinating the turnings on locusts [49, 50]. We believe the stable and efficient flight cannot be separated from the diverse auxiliary manoeuvres. Unfortunately, as the outstretching of forelegs is not commonly seen on flying insects other than beetle, their roles have not been systematically studied yet. Inspired by the above studies, experiments were carried out on verifying the hypothesis that forelegs are purposely outstretched for assisting the flight.

In this work, we conducted leg motion measurement and electromyography (EMG) measurement on leg muscles under visual stimulations. By recording the angular displacement of the forelegs, we found that both legs swung clockwise under left visual stimulation and counterclockwise under right visual stimulation. Consistently, the EMG measurements of leg protraction muscle proved that more spikes happened on the ipsilateral side with stimulation's direction whereas less spikes happened on contralateral side. According to the motion patterns under visual stimulations, we believe the usage of forelegs is for the angular momentum generated by the leg swing. By electrically eliciting leg motions, the generated torque was measured to estimate the effect in flight based on angular momentum. An angular change of $\sim 2^\circ$ was calculated

on the body, which is remarkable enough in flight control. Apart from the tethered tests, we conducted free flight tests with custom designed backpack. Electrical stimulations on leg muscles could be applied from the backpack in flight. It is found that the stimulated leg motion could deflect the flight course in accord with the direction of the visual stimulation under same motion. As the wings are the dominant mechanism for controlling insect flight [5], this study indicates that the outstretched foreleg plays a supplemental role in steering during flight.

6.2: Foreleg Motions under Visual Stimulation

Once a beetle starts to fly, whether in free or tethered flight, its forelegs extend (Figure 6.1). We hypothesized that the unique posture of beetle may have some effects on its flight control, especially on the turning control. Thus, we conducted leftward and rightward visual stimulation of tethered beetles using optical flow of dark and bright stripes to induce fictive turns [141]. To study the correlation between turning and foreleg motion, we tracked foreleg motion during visual stimulation using a 3D motion capture system (Vicon, Figure 3.4). During the fictive turns, the horizontal swinging of extended forelegs was frequently observed (Figure 6.2a). Within a sample size ($N = 10$ beetles, $n_{\text{left}} = 80$ left fictive turns, $n_{\text{right}} = 80$ right fictive turns), we found that the forelegs mostly swung clockwise (from the view of the beetle's dorsal side) to produce fictive left turns and counterclockwise to produce fictive right turns (Figure 6.2b). All clockwise and counterclockwise rates showed a significant difference from chance level ($p < 0.0001$, binomial test).

Figure 6.2. Angular displacement of forelegs in response to visual stimulation.

(a) Representative angular displacements were synchronized with visual stimulations. The black lines show the angular displacements of left and right legs (θ_{left} and θ_{right}). The green and red bars show the average angular displacement in response to left and right visual stimulation, respectively. The length of the bars indicates the stimulation period. During left stimulation, the left leg moved closer to the body and the right leg moved away from the body (clockwise). During right stimulation, both legs moved in the opposite direction (counterclockwise). (b) The occurrence rates of clockwise (CW) and counterclockwise (CCW) swings were determined for both forelegs ($N = 10$ beetles, $n_{left} = 80$ left stimulations, $n_{right} = 80$ right stimulations). The results revealed that the left leg (b1) and right leg (b2) moved clockwise at 83.8% and 80.0%, respectively, during left stimulation, whereas they moved counterclockwise at 86.3% and 78.8%, respectively, during right stimulation.

We speculated that the leg swings associated with fictive turns were actively induced by the beetles itself, i.e., visually stimulated beetles exhibit optomotor responses, including the activation of leg muscles, to produce swinging. To clarify whether the leg swings were produced actively or passively, we used electromyography (EMG) to assess the protraction muscle of the left leg (Figure 6.3a) during visual stimulation. The contractions of the muscle in the left foreleg cause it to swing clockwise [100]. Indeed, one-sample t-test indicated the majority of EMG spikes in the muscle occurred during left fictive turns (Figure 6.3c $N = 5$ beetles, $n = 5$ EMG recordings, $t(4) = 10.82$, $p = 0.0002$), whereas minority spikes occurred during right stimulation ($t(4) = 8.44$, $p = 0.0006$). This result suggests that the foreleg swings during fictive turns are not induced by external forces but rather the tension created by leg muscle contraction.

Figure 6.3. EMG measurements of the protraction muscle of the left foreleg.

(a) Anatomical view of the left leg protraction muscle. (b) Representative EMG from protraction muscle during tethered flight. The recorded signals were synchronized with visual stimulation. The green and red bars indicate the periods of left and right visual stimulation, respectively. (c) The occurrence rate of EMG spikes during visual

stimulation was determined (N = 5 beetles, n = 5 EMG recordings). In the protraction muscle, 54.1% of the spikes occurred during left visual stimulation, whereas only 14.8% occurred during right visual stimulation. The error bars represented the standard deviation.

6.3: Analysis on the Torque Induced by Foreleg Swing

The yaw torque exerted on a beetle's body during a leg swing effectively rotates the body. We tethered a beetle on a torque meter and electrically stimulated the protraction muscle of the left foreleg to produce clockwise swinging (N = 4 beetles, n = 40 trials). The muscular reaction induced by electrical stimulation is fast and obvious, which does not reveal time delay [35, 100]. It is known that the pitch angle mainly correlates with the longitudinal flying speed and does not apparently influence on the horizontal turning on flapping wing flyers [89, 142]. Thus, we focused on the analysis of yaw torque and roll torque. The results revealed that the induced torque on yaw was as much as $\sim 7 \mu\text{N m}$ whereas the induced torque on roll was relatively small, which was $\sim 2 \mu\text{N m}$ (Figure 6.4 a1,b1). Meanwhile, the moment of inertia estimated from the beetle model (Figure 3.7) is 12.36 g cm^2 in yaw and 7.73 g cm^2 in roll. Thus, it could be calculated that the induced angular displacement was 1.62° on yaw and 0.69° on roll within 200 ms. The same experiment and analysis were repeated after removing the legs from the body. The measured torque was apparently smaller, indicating that the significant torque observed prior to leg removal was solely due to the foreleg swinging (Figure 6.4 a2,b2, N = 4 beetles, n = 40 trials).

Figure 6.4. The induced torque generated by electrical leg stimulation.

The induced yaw torque (**a**) and induced roll torque (**b**) on the body, ΔT_{yaw} and ΔT_{roll} , was measured on both intact (**a1** and **b1**) and amputated (**a2** and **b2**) beetles by stimulating the left foreleg with 0.7 V electrical pulse signals. The black solid line represents the average induced torque, and the gray shaded area indicates the standard deviation ($N = 4$ beetles, $n = 40$ trials). The blue lines represent the calculated body angular velocities based on the induced torques.

Moreover, a simulation was conducted to compute the turning rate of trajectory based on the angular velocity of the body. In a flight turning induced by a yaw torque, the angular displacement in body orientation does not equal to the angular displacement in flight trajectory because the centrifugal force of the turning is provided by the misaligned horizontal propulsion, which can be expressed as follows:

$$m \cdot v \cdot \omega_{traj} = F_h \cdot \sin(\theta),$$

where m is the weight of beetle, v is the horizontal flight velocity, ω_{traj} is the turning rate of trajectory, F_h is the horizontal propulsion, and θ is the included angle between body orientation and flight trajectory. As the leg-induced angular displacement is $\sim 1.6^\circ$ in body orientation, the included angle should be even smaller. Then the equation can be transformed as follows:

$$m \cdot v \cdot \omega_{traj} \approx F_h \cdot \theta = F_h \cdot \left(\int \omega_{body} - \int \omega_{traj} \right),$$

where ω_{body} is the angular velocity of body. Thus, we know the angular velocity of body is not the same with the turning rate of trajectory. Furthermore, the horizontal propulsion was measured by the torque meter from tethered flying beetles, which was 0.131 ± 0.014 N ($N = 5$ beetles, $n = 50$ measurements; mean $\pm$ SD). As shown in Figure 5.1, the average flight velocity of beetle with backpack loaded was 5.57 m s^{-1} . The 6th order polynomial curve fitting was carried out to approximate the induced angular velocity in Figure 6.4a1. Thus, the corresponding turning rate of the flight trajectory induced by yaw torque could be solved and the value was $3.84^\circ \text{ s}^{-1}$ at the timing of 150 ms after the beginning of stimulation, which was close to the duration of electrically induced leg swing.

6.4: The Effect of Foreleg Muscle Stimulation in Flight

As further support of the role of forelegs in flight, we demonstrated that exogenous stimulation of the leg muscles induces foreleg swinging and subsequent turning while in free flight (Figure 3.13). A radio-controlled backpack (Figure 3.11) was mounted on the beetle, and the protraction muscles of the left and right forelegs were alternatively

stimulated to induce the clockwise swing of the left foreleg and the counterclockwise swing of the right foreleg, respectively. As expected during free flight, the beetle turned left when the left foreleg was stimulated to swing clockwise, and turned right when the right foreleg was stimulated to swing counterclockwise (Figure 6.5 c1,d1, $N = 5$ beetles, $n_{\text{left}} = 162$ left stimulations, $n_{\text{right}} = 184$ right stimulations, $p < 0.0001$, binomial test). Due to the electrical stimulation of left or right foreleg protraction muscle, the mean incrimination for the turning rates was $3.29 \pm 12.71^\circ \text{s}^{-1}$ and $-5.42 \pm 11.57^\circ \text{s}^{-1}$ (mean $\pm$ SD), respectively (the positive or negative value indicates that the turning rate for left or right turns increases, respectively). We confirmed from the preliminary test that the electrical stimulation of the protraction muscles solely induced leg swinging and did not affect flight muscles (Figure 3.14). Interestingly, once the forelegs were removed from the beetle, left and right turns occurred at a similar rate regardless of which side was stimulated (Figure 6.5 c2,d2, $N = 5$ beetles, $n_{\text{left}} = 90$ left stimulations, $n_{\text{right}} = 90$ right stimulations) with a mean rate of $-0.38 \pm 12.09^\circ \text{s}^{-1}$ and $-2.35 \pm 9.60^\circ \text{s}^{-1}$, respectively (mean $\pm$ SD). According to t-test, the difference in turning rates between intact and amputated beetles is considered statistically significant under both left ($t(250) = 2.23$, $p = 0.013$) and right ($t(272) = 2.18$, $p = 0.015$) electrical stimulations. Thus, the left-turn and right-turn shown in Figure 6.5b were generated by the swinging motion of the left or right foreleg.

Figure 6.5. Results of the foreleg stimulation in free flight.

(a) Overview of the backpack-mounted beetle. (b) A zigzag flight path was produced by electrical stimulation of the left foreleg protraction muscle to produce left turns and vice versa. The electrical stimulation of the left or right protraction muscle induced the swing of the corresponding leg clockwise or counterclockwise (viewed from the dorsal side of the beetle). Segments in green and red indicate the trajectories during left and right stimulation, respectively. The occurrence rates of left and right turns during electrical stimulation were determined before (c1) and after (c2) the removal of the legs. Prior to leg removal, left stimulation induced left turns at a rate of 67.9% and right stimulation induced right turns at a rate of 70.7% ($N = 5$ beetles, $n_{\text{left}} = 162$ left stimulations, $n_{\text{right}} =$

184 right stimulations). After the legs were removed, the occurrence rate was approximately 50% regardless of which side was stimulated ($N = 5$ beetles, $n_{\text{left}} = 90$ left stimulations, $n_{\text{right}} = 90$ right stimulations). Histograms of induced turning rates before (**d1**) and after (**d2**) leg removal were presented (negative sign denotes turning toward the right). The green and red dashed lines represent the polynomial fitted distribution of induced turning rates during left and right stimulation, respectively.

6.5: Discussions and Conclusions

In flight, many insects fold their forelegs tightly close to the body, which naturally decreases drag or air resistance, whereas flying beetles stretch out their forelegs for some reason. We hypothesized that the forelegs are “intentionally” outstretched and swung to facilitate turning while in flight and that the beetle turns its body orientation (the direction of propulsion) by swinging its forelegs. To test this hypothesis, we used kinematic and physiological analyses to determine if swinging the legs during flight was regulated, and we measured the torque exerted on the body during leg swinging using tethered beetles. Furthermore, we induced left and right turns in freely flying beetles by electrically stimulating their leg muscles to produce a swinging motion. This stimulation was achieved by mounting our custom designed miniature wireless communication device (remote stimulator backpack) on flying beetles.

Through visually inducing fictive turns on tethered beetles, we revealed that the forelegs showed certain swinging motions in accord with the directions of the visual stimulations. Specifically, the beetle voluntarily swings the forelegs clockwise or counterclockwise while in flight to turn in the opposite direction. By monitoring the EMG signals from the left foreleg protraction muscle, whose activation generates a clockwise swing of the left foreleg, we found that the spikes appeared much more frequently in the left visual

stimulations than in the right stimulations, which gives the evidence that the swinging motions of forelegs were actively induced by the tension created by muscle contraction rather than passively from some external forces. Thus, beetles voluntarily manipulate their forelegs to produce fictive turns in response to visual stimulation.

Meanwhile, the torque measurement proved that a leg swing apparently induced yaw torque on a beetle's body, which would lead to an angular displacement in yaw up to 1.62° within 200 ms. The finding implies that the torque generated by leg motion is significant enough to rotate the heading direction of beetle. Since left foreleg was stimulated to swing clockwise in the experiment, the body tended to rotate counterclockwise as a consequence. The rotating direction of the body is in accordance with the observations in visual stimulation. As a comparison, the induced roll torque would generate a 0.69° roll displacement within 200 ms. As a linear relationship between roll angle and yaw angular velocity was found on beetle [143], the roll displacement (0.69°) would correspond to a $-2.29^\circ \text{s}^{-1}$ angular velocity in yaw, which is apparently smaller than the effect of yaw torque ($\sim 13^\circ \text{s}^{-1}$; Figure 6.4a). Moreover, the yaw angular velocity induced by clockwise leg swing rotated the body leftwards, which was in accordance with the direction of visual stimulation when forelegs were swinging clockwise. Thus, the leg swing in the turnings should be employed for the yaw-turn rather than bank-turn. In flight, the viscous forces acting on bodies are remarkable on small insects whereas the inertia forces are dominant on large insects [43]. Specifically, the Reynolds number of a small fruit fly in hovering is approximately 150 [105]. The Reynolds number of a flying hawkmoth is as large as approximately 5500 [89]. Since we are using a kind of large beetle, the inertia forces will be far more prominent than the viscous forces. The use of body posture to change the flying direction by exerting

additional torque on the body was already reported on insects [56, 85]. Thus, we believe the beetle swings the forelegs clockwise or counterclockwise in order to generate a yaw torque to rotate its heading direction towards the fictive turns.

The aforementioned findings from the tethered experiments verified our hypothesis that forelegs are swung to facilitate turning. As further support of our hypothesis, we demonstrated that exogenous stimulation of the leg muscles induces foreleg swinging and subsequent turning while in free flight. In accord to the results of the tethered experiments, the swinging of left leg (clockwise) induced a leftward turning and the swinging of right leg (counterclockwise) induced a rightward turning (Figure 6.5c1). After the leg amputation, the leftward and rightward turnings showed negligible difference in occurrence rate (Figure 6.5c2). The electrical stimulations were exactly applied on the leg protraction muscles without influencing any flight muscles (Figure 3.14). The results tell that the induced turnings are absolutely because of the swinging motion of forelegs. According to the simulation result, the induced angular velocity estimated from the torque measurement well matched the induced angular velocities calculated from the free flights. Together with the directional agreement, we further proved that the turning induced by leg motion was due to the generated torque. As known from visual stimulation that forelegs are voluntarily swung in turnings, we demonstrate that beetles manipulate their forelegs as a mechanism of flight directional control. However, the induced turning rates are relatively small. We understand that the range of leg swing is constrained by structural limit of the leg coxa and the leg motion cannot sustain a large flight turning. Actually in a sharp or long-lasting turning, we believe the mechanism in charge is not the legs but the wings. Compared to the wings, the leg motion reveals its advantages in response time and precision. We believe that the

leg is a supplementary mechanism to generate small directional corrections or initiating a flight turning in flight.

The wings, as well as their articulations and flight muscles, are the undeniable dominant mechanism for controlling insect flight [1, 5, 35, 76]. The operating principles of wings have been well studied in various insects [56, 99]. In fact, beetles flew well even after their legs were removed. However, this study indicates that the outstretched foreleg plays a supplemental role in steering during flight. Auxiliary flight control by body parts other than wings has been established in some insects [50, 52, 54, 55, 57, 59, 62, 84, 85, 90]. For example, flies, locusts, and honeybees can sway or twist their abdomen to facilitate turning during flight or change their body posture to adjust their flight speed [52, 59, 62, 84, 90]. The abdomen of a beetle is relatively rigid and short; thus, it maybe not feasible or effective to manipulate the abdomen as a method of steering control during flight [144, 145]. The forelegs of beetles are relatively large, wide, and thick when compared with other insects (Figure 6.1); however, this design may not be primarily for steering while flying but rather for direct uses, such as dirt digging. If these large legs were folded closely to the body, they would be useless in flight. Instead, the beetles outstretch and swing their forelegs to facilitate turning during flight.

In summary, we found that the forelegs of beetle *Mecynorrhina torquata* were swung clockwise during left turns and vice versa, and the leg muscles were fired accordingly to elicit the swing. In addition, swinging the legs generated significant torque on the beetle body. Furthermore, flying beetles were remotely radio-controlled to perform left–right turnings by electrically stimulating the leg muscles via the miniature radio device. The results and demonstration reveal that the beetle’s forelegs play a supplemental role in directional steering during flight. Collectively, we believe future studies on the effect of

wings and other non-wing body parts on flight should clarify insect flight mechanisms and provide novel insights for the design of insect-scale robotic flapping flyers.

Chapter 7 : Conclusion and Future Works

7.1: Conclusion

In the thesis, we demonstrated our study of flight initiation by DLM stimulation, banked turn in flight measured by IMU and roles of forelegs in flight steering.

According to the results of flight initiation experiment, beetle flight could be initiated by a simple method, which is applying electrical pulse signals to DLMs, with high success rate, mild damage extent and low power consumption. Meanwhile, as the wing-beat principles and muscular activities of many insects are quite similar, in which the down- and up-stroke of the wing is driven by the alternating actions of DLM and DVM respectively, our approach could make significantly contributions to the flight initiation on other insects as well. We believe the study on flight initiation will help the future design of insect-machine hybrid MAVs. Moreover, we wish the technologies and knowledge of electrical stimulation of living muscles can help human beings on the functional electrical stimulation (FES), a technique to electrically activate nerves innervating extremities affected by paralysis to induce desired motor actions and behaviors of the patient.

The difficulty of recording and stimulating neural and muscular sites during untethered flight has been a long-standing hurdle for studies of insect flight. We custom-designed an IMU-integrated backpack suitable for mounting on flying insects, and hence measured the body attitudes on flying beetles. The IMU was sufficiently accurate to track the angular maneuvers of the freely flying beetles. The low weight and small size of the IMU backpack enable remote radio recordings without affecting the flight behavior. Through the free flight experiment, we found a strong coupled correlation between the roll angles and yaw angular velocities. The

correlation coefficients proved the existence of coupling between the two variables. Moreover, the average yaw velocities were linearly correlated with the roll angles. However, beetle can actively manipulate the body roll rotation with a ~60 ms time gap ahead of yaw rotation implies a non-coupled roll control. Moreover, the roll angular velocities fluctuated, suggesting active adjustment of the roll angle. The designed IMU backpack should be useful for unravelling the mechanism and principle in flapping flight of the insects.

According to our study of foreleg motions in flight, the role of forelegs in steering can be concluded. It is found that beetles always stretch out their forelegs during flight and the forelegs show certain motion patterns following the visual stimulations. Specifically, the forelegs were swung clockwise during left fictive turns and counterclockwise during right fictive turns. It was found the leg muscles were fired accordingly to elicit the swing purposely. In addition, flying beetles were remotely radio-controlled to perform left–right turnings by electrically stimulating the corresponding leg muscles. Thus, we believe that flying beetles voluntarily stretched out and swung their forelegs to assisting flight turning by rotating their bodies in the direction opposite to the leg rotation. These findings may inspire the improvement of flight controllability and stability on insect–machine hybrid MAVs.

7.2: Future Works

Our experimental results have demonstrated a reliable and safe method to initiate the wing flapping on beetle. The relationship between roll angle and yaw angular velocity in the banked turn was summarized with an insect-body-mountable IMU. A wireless electrical stimulator was used to elicit demanded muscle contractions in

free flight. With the advantage of experimental equipment, more and more secrets in insect flight have been discovered, which lead us closer to the insect-machine hybrid system. However, there are still unsolved problems obstructing us from achieving the insect-machine hybrid system. Towards solving these problems, the following issues are set as the recommendations for future works in this study:

- A flying insect may have as many as 13 direct flight muscles. The functions of many flight muscles have been well established. Normally, these muscles were studied and evaluated separately from other flight muscles. However, multiple muscles were found to collaborate to make different performances in flight, which is still a challenge awaiting exploration [4]. Thus, we would like to explore the cooperation between two or more muscles in flight and compare their collaborated effects with either muscle alone.
- As we could use the IMU backpack to collect body attitudes in flight, the roll angles of insect body and the relationship between roll angles and banked turn were well summarized. Similar with roll angle, the pitch angle is frequently and clearly manipulated in flight as well. Thus, the IMU backpack can be used to measure the pitch maneuver in flight and analyze its effect on flight direction or velocity.
- Currently we have successfully demonstrated the flight control by electrical stimulation on muscles. The pulse signals were generated from a mounted backpack, which was manually commanded and the current flight attitude was not considered for stimulation. Thus, the idea of combining the wireless stimulator and IMU onto a single backpack came to our mind. Together with

the studies on roll angle and pitch angle in flight, we will be able to optimize or adjust the stimulation protocol based on the body attitudes of the insect.

- In order to achieve the insect-machine hybrid system, we need to demonstrate the reliable and accurate flight trajectory control. A motion capture system will be needed to track the coordinates of the flying insect. Moreover, a feedback flight control system will be required to generate stimulation commands timely and consecutively. The stimulations are modulated to force the insect to follow a predetermined path. Even though there are still a lot of uncharted mechanisms or principles in insect flight control, our attempts on the flight trajectory control will navigate our research direction towards the insect-machine hybrid system.

List of Publication

Journal papers

- [1] H. Y. Choo, Y. Li, F. Cao, H. Sato, “Electrical Stimulation of Coleopteran Muscle for Initiating Flight”, *PLoS ONE*, **11(4)**, e0151808, (2016), (**Q1, IF = 2.806**).
- [2] Y. Li, F. Cao, T. T. Vo Doan, H. Sato, “Controlled banked turns in coleopteran flight measured by a miniature wireless inertial measurement unit”, *Bioinspiration & Biomimetics*, **11 (5)**, 056018, (2016), (**Q1, IF = 2.939**).
- [3] Y. Li, F. Cao, T. T. Vo Doan, H. Sato, “Role of outstretched fore legs of flying beetles revealed and demonstrated by remote leg stimulation in free flight”, *Journal of Experimental Biology*, **220(19)**, 3499-3507, (2017), (**Q1, IF = 3.320**).
- [4] Y. Li, J. Wu, H. Sato, “Feedback control-based navigation of a flying insect-machine hybrid robot”, *Soft Robotics*, published online, (**Q1, IF = 8.649**).
- [5] F.Cao, C. Zhang, T. T. Vo Doan, Y. Li, D. H. Sangi, J. S. Koh, N. A. Huynh, M. F. Bin Aziz, H. Y. Choo, K. Ikeda, P. Abbeel, M. M. Maharbiz, H. Sato, “A Biological Micro Actuator: Graded and Closed-Loop Control of Insect Leg Motion by Electrical Stimulation of Muscles”, *PLoS ONE*, **9(8)**, e105389, (2014), (**Q1, IF = 2.806**).
- [6] C. Zhang, F. Cao, Y. Li, H. Sato, “Fuzzy-controlled living insect legged actuator”, *Sensors and Actuators A: Physical*, **242**, 182-194, (2016), (**Q2, IF = 2.499**).

International Conferences

- [1] T. T. Vo Doan, **Y. Li**, F. Cao, H. Sato, “Cyborg beetle: Thrust control of free flying beetle via a miniature wireless neuromuscular stimulator”, *28th IEEE International Conference on Micro Electro Mechanical Systems (MEMS)*, pp. 1048–1050, Estorial, (2015), (**Acceptance Rate = 41 %**).

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