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**RPPR Final Report**  
as of 03-Aug-2018

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**STEM Degrees:**

**STEM Participants:**

**Major Goals:** In this 1-year, short-term investigatory research project we aimed to collect initial data on the dynamics of legged locomotion for small-scale running ants. The goal of this data collection effort is to better distinguish the dynamics of running across body size scaling. In our proposed work we aimed to carry out three areas of locomotion study: (1) develop a force sensor to study foot contact mechanics during running, (2) develop a high-throughput multi-camera imaging suite for observing ant locomotion, and (3) establish robust experimental protocols to observe repeated and consistent locomotion behavior of ant colonies. This funding supported the salary of a postdoctoral scholar for a 9-month period and subsequent salary support has been provided through UCSD funding and PI Gravish's startup funds.

**Accomplishments:** Please see uploaded PDF

**Training Opportunities:** A postdoctoral scholar was hired and mentored during this project. Please see uploaded PDF for more information.

**Results Dissemination:** Please see PDF for more information.

**Honors and Awards:** Please see PDF for more information.

**Protocol Activity Status:**

**Technology Transfer:** None. Please see PDF for more information.

**PARTICIPANTS:**

**Participant Type:** Postdoctoral (scholar, fellow or other postdoctoral position)

**Participant:** Glenna Clifton

**Person Months Worked:** 12.00

**Funding Support:**

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

**RPPR Final Report**  
as of 03-Aug-2018

## **Final report for ARO-STIR: Dynamical templates for sub-milligram legged locomotion**

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### **Problem studied and project overview**

In this 1-year, short-term investigatory research project we aimed to collect initial data on the dynamics of legged locomotion for small-scale running ants. The goal of this data collection effort is to better distinguish the dynamics of running across body size scaling. In our proposed work we aimed to carry out three areas of locomotion study: (1) develop a force sensor to study foot contact mechanics during running, (2) develop a high-throughput multi-camera imaging suite for observing ant locomotion, and (3) establish robust experimental protocols to observe repeated and consistent locomotion behavior of ant colonies. This funding supported the salary of a postdoctoral scholar for a 9-month period and subsequent salary support has been provided through UCSD funding and PI Gravish's startup funds.

### **Key personnel**

STIR funding enabled the hiring of a postdoctoral scholar (PD) to perform project work and mentor graduate students. Through a round of interviews the PD, Glenna Clifton, was selected as the optimal candidate for the position. Dr. Clifton received her PhD from Harvard University in the department of Organismic and Evolutionary Biology with Dr. Andy Biewener. During her PhD, Dr. Clifton studied the fluid mechanics of bird foot propulsion within water and on water surfaces, using a suite of physics theory, high-speed imaging, and robotics experiments to address these questions. Dr. Clifton's undergraduate degree is in physics and thus her multidimensional skill-set in organismal biology and physics made her an ideal candidate for the position.

### **Summary of results**

Here we summarize the results from the three project efforts described above. Early on in the project we determined that the primary goal of observing legged locomotion dynamics would have the most success through aims (2) and (3) and thus the majority of project work was focused on these tasks. This project resulted in two conference presentations by Dr. Clifton (Society of Integrative and Comparative Biomechanics, and Dynamic Walking) and no patents or inventions. A publication from this funding is in process.

#### *(1) Contact mechanics of foot ground interactions*

We proposed to measure ground reaction forces of running ants by fabricating a custom force sensor. Prior to working on the force sensor we determined that two optical techniques would provide similar information about foot-ground contact mechanics without requiring significant mechanical design and fabrication efforts. For aim (1) we performed pilot experiments with two experimental setups: (i) a total internal reflection substrate demonstrating foot contact area in real-time, and (ii) a traction force microscope used in an adjacent lab at UC San Diego.

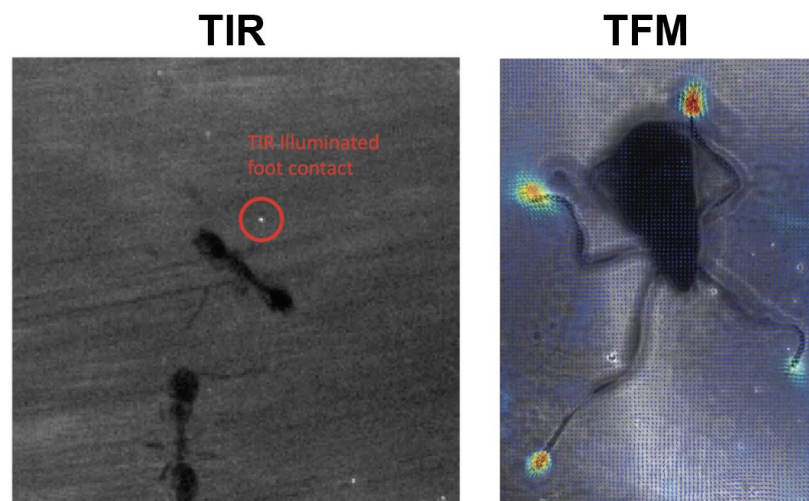
In the total internal reflection set-up (TIR), light travels through a sheet of glass with only structures within nanometer distances from the surface scattering the light. Thus TIR can directly observe the true contact area between two surfaces. Ants such as the Argentine ant (*Linepithema humile*) used in these studies can actively control soft, adhesive pads on the underside of their feet which may enable large shear forces during locomotion. We sought to develop TIR as a method for real-time contact area observation.



**Figure 1. Two measurement techniques explored for contact mechanics observation of small-mass runners.**

Traction force microscopy (TFM) uses fluorescent microparticles embedded in an elastic polymer layer to measure shear and normal contact stresses of small-scale organisms. By focusing a microscope on the fluorescent particles we can track particle displacement under foot contact stresses and with information about the elasticity of the polymer layer we can calculate contact stress.

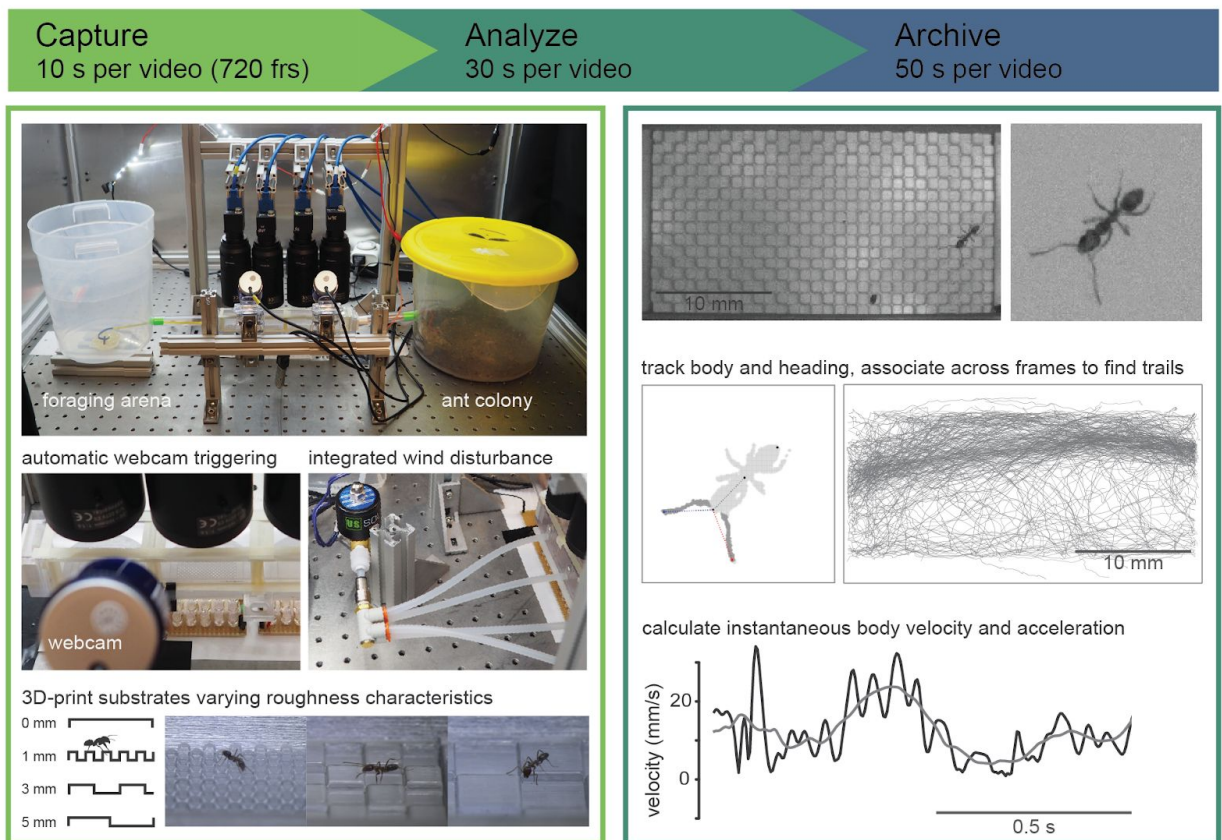
Both TIR and TFM methods were attempted in the first months of the project. We determined that each method is capable of visualizing foot contact mechanics of ants (Fig. 2), however each method suffered initial limitations that eventually lead us to pursue the more fruitful methods described in tasks 2 and 3. TIR measurements are still ongoing being performed by a graduate student funded through the PIs startup.



**Fig. 2. Example results from TIR (left) and TFM (right) pilot experiments.**

## (2) Multi-camera high-throughput imaging techniques

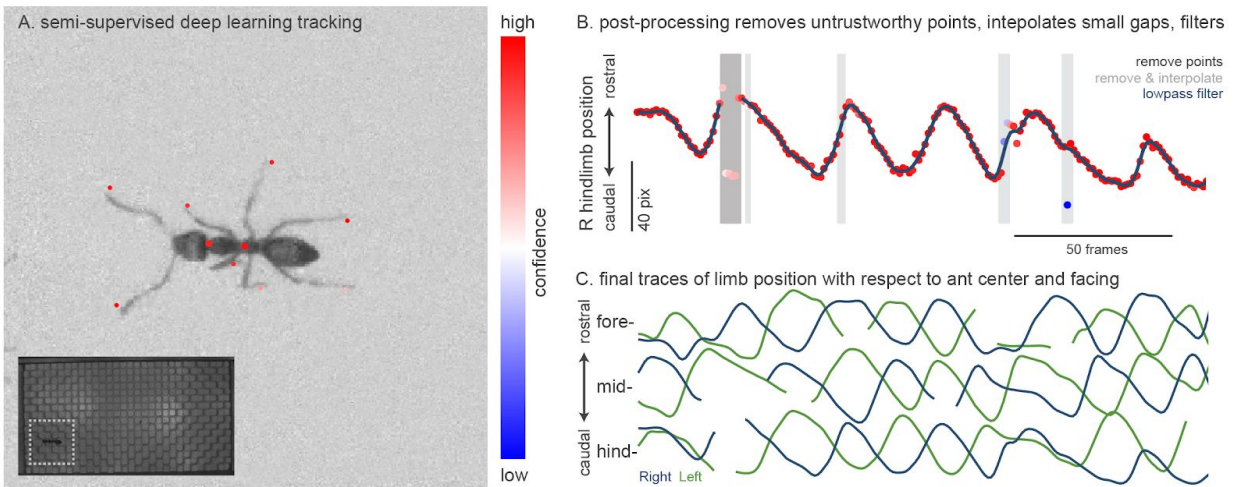
In aim (2) we set out to develop a multi-camera system that could automatically capture and analyze running bouts from ants traversing level, rough, and sensor-laden substrates. Fig. 3 shows the workflow we established for this aim. Four high-speed cameras, triggered using lower speed webcams, record an enclosed tunnel containing 3D-printed substrates that can be varied and interchanged. We added to this system an automated “scented” air inlet to excite ants to maximal running speeds so that we can look at speed distributions in quiescent and perturbed states. The goal of this experiment is to understand the distribution of running kinematics at normal and at maximal speeds, across a variety of substrates.



**Fig. 3. Overview of the capture, analyze, and archive method developed for high-throughput imaging of ant locomotion. A four-camera system monitors ants moving through an enclosed tunnel (left). We measured locomotion on smooth and rough substrates to observe how ground complexity influenced running dynamics. Automated image processing extracted the approximate center of mass kinematics of moving ants as well as antennal positions (right).**

To further improve the kinematics information gathered in pilot experiments on rough and smooth substrates we implemented a pre-print deep-learning method for limb automated tracking (<https://github.com/talmo/leap>). Dr. Clifton was able to integrate the deep-learning

tracking into our automated analysis pipeline. The neural-network was trained with hand-tracked data then used to resolve limb kinematics with fairly good accuracy (> 80% confidence). Fig. 4 displays example tracking of the limbs and antennae on a 1mm substrate. Notably, on the rough 1 mm substrate, the limb periodicity and phasing are perturbed consistently

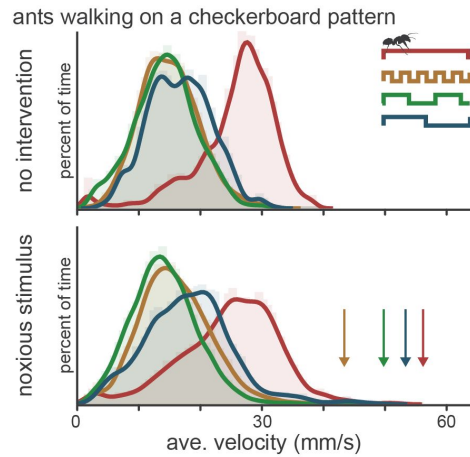


**Fig. 4. Processed and raw image from high-throughput locomotion experiment (left). Red markers indicate automatically tracked points on feet. Points at limb joints are also tracked but not shown. Upper right shows confidence estimates from tracking a single limb, gray bars indicate regions removed or interpolated. Bottom right shows all six limbs tracked automatically with missing data illustrating frames with low confidence.**

### (3) Experimental procedures for repeated locomotion observation

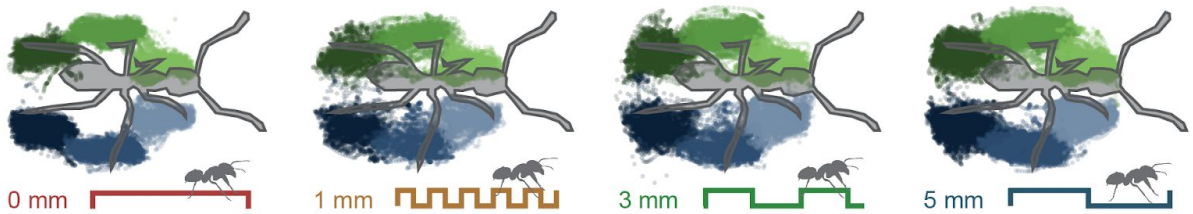
Following setup of an automated capture and analysis system for legged locomotion studies we set out to collect center of mass and limb kinematics observations across terrains with varying roughnesses. In this last task we collected Argentine ant (*Linepithema humile*) workers in quantities of >300 individuals at a time from collection sites distributed around the UC San Diego campus. Ants were housed in an enclosed nest-like chamber with soil. This chamber had a single outlet through the observation tunnel to a foraging arena in which food and water were provided. After freely exploring the arena, ants would quickly establish a foraging trail through the tunnel (can be seen in Fig. 3, left middle image of ant tracks). By setting up experiments to motivate ants into a foraging recruitment process, we are able to collect thousands of repeated locomotion observations from a single colony. In controlled experiments we tested nine colonies across four substrates (smooth and 1mm, 3mm, 5mm checkerboards). After removing three colonies due to experimental quality issues we have recorded over 10,800 videos, corresponding to over 13,000 observations of ants walking across the substrates. Figure 5 shows speed distributions across the four substrates with and without the noxious gas stimulant. Distributions show that all three rough substrates resulted in slower running speeds. However, when motivated to sprint by a noxious gas stimulus, ants were able to attain very high-speeds (though infrequently) on all substrates (Fig. 5, bottom arrows). These results

suggest that speed regulation on the different substrates may not be a biomechanical limit but instead a preference.



**Fig. 5. Speed distributions with and without a noxious gas stimulant (top and bottom). Color denotes substrate roughness with the legend in the upper right corner. Arrows in bottom plot indicate velocity extremes for the four substrates.**

The last analysis completed during the project duration examined body-centric variation in foot placement as a function of substrate (Fig. 6). The point clouds represent the location of the limbs during running bouts. There is a clear trend of increased variance in foot placement between smooth (far left) and rough substrates. The 1mm substrate demonstrated the most foot placement variance suggesting that ants are consistently perturbed in foot placement and potentially leg phasing on these substrates. Despite this variation, ants are capable of high-speed running on even the toughest substrate (1mm), suggesting that ants may differentially coordinate limb placement and limb control on rough substrates to achieve effective, and fast running locomotion.



**Fig. 5. Point clouds representing foot position during running bouts on four substrates.**

### Conclusion

The project work supported by the STIR has generated significant and exciting preliminary data towards better understanding the complexity and dynamics of legged locomotion. Specifically, at small scales organisms are presented with highly rugose substrates yet also have high limb strength-weight and thus optimal legged dynamics in small scale systems may be very different than large scale systems. The automated methods developed here and the social insect study system combined enable us to perform large scale observations of locomotion behavior and better understand behavioral and stochastic variation in locomotion control.