

Laser Biospeckle in Speedy Investigation of Seed Germination under Simulated Microgravity

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1. Introduction

Seed germination is a critical phase in the plant life cycle, influencing vegetation establishment under various environmental conditions. Understanding the effects of altered gravity on germination is vital for advancing agriculture in extra-terrestrial environments, such as space habitats or Martian bases. Microgravity studies also reveal fundamental insights into gravity perception and adaptation in living organisms. To simulate microgravity, clinorotation is commonly used, providing near-weightless conditions through controlled rotation.

In this study, we propose using laser bio speckles to compare germination under microgravity conditions. Laser bio speckles are generated when scattered light from structures within the seed interfere, and the intensities of such speckles change in time, and these changes reflect changes in the scattering structures within the seed. We have already demonstrated its effectiveness in plants [1].

To stimulate microgravity, we varied the rotation speeds from stationary conditions to two rotation speeds 3 rpm and 7 rpm. We tested the proposal using garden cress (*Lepidium sativum*) and lentil seeds (*Lens culinaris*), a widely cultivated legume renowned for its resilience and rapid germination. We compared the results of laser bespeckles under stimulated microgravity of different species.

2. Experiments and results

Figure 1. illustrates the experimental system. A laser diode emitting light at a wavelength of 638 nanometers illuminates the sample. The light scattered from the sample interferes to produce speckles on the plane of a CMOS camera (ThorLabs DCC1545/1645, New Jersey, USA). The speckle videos were acquired by a CMOS camera connected to a computer for further analysis and processing. The presence of an aperture and the use of a mirror provide flexibility in directing and focusing the laser beam, allowing for precise control over the illumination and observation of the sample.

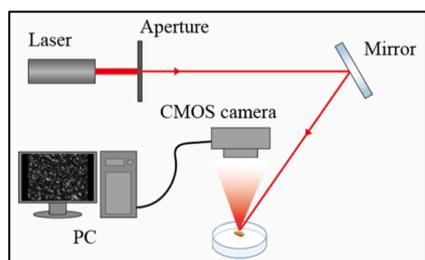


Fig.1. Schematic diagram of the experimental system

In the experiment, garden cress seeds (*Lepidium sativum*) were exposed to simulated microgravity conditions using rotation speeds of control and 9rpm on a clinostat for 24 hours. Biospeckle measurements were taken before and after the rotation to evaluate seed dynamics. Cross-correlation analysis was performed by comparing the reference frame (the first frame of the recording) with subsequent frames, and the correlation time—defined as the point where the cross-correlation coefficient decreases to $1/e$ —was used to quantify seed movement patterns and metabolic activity.

Figure 2(a) illustrates the cross-correlation under two experimental conditions (0rpm and 9rpm) at three different times. A difference in trend of decreasing correlation with increasing time lag is observed for both conditions, indicating the activity differences with respect to the rotating speeds. For the 9rpm condition distinct oscillations in the autocorrelation function, particularly at longer time lags, indicating the presence of additional periodic or rhythmic components in the signal compared to the 0rpm condition. This suggests that the 9rpm condition may induce rhythmic fluctuations in the signal that are not present in the g condition.

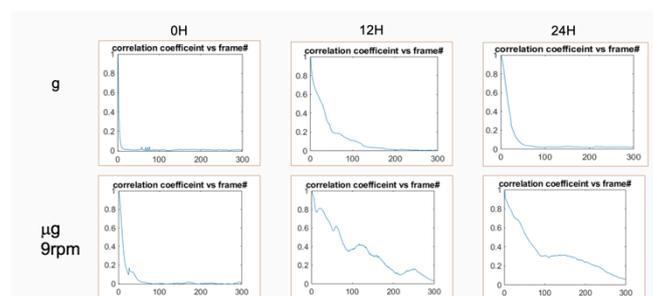


Fig.2(a). Correlation between Control and clinorotated seeds.

Figure 2(b) presents the correlation coefficient as a function of measurement time in hours. The correlation time for the control condition indicated relatively consistent and slow movement of the seeds. However, as the rotation speed increased to 9rpm, a moderate increase in the correlation time was observed, suggesting a noticeable change in the seed movement pattern due to the higher rotational speed. The most significant increase in correlation time occurred at 24 hours, where the correlation time reached its peak, indicating a substantial increase in seed movement speed and longer correlation lengths. We are currently investigating the effects for lentil seeds and the specie differences.

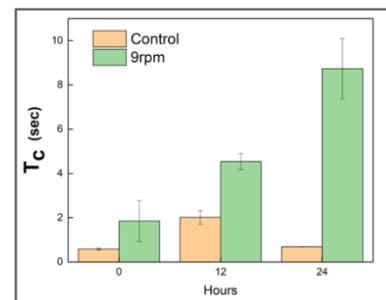


Figure 2(b). Time taken to reach 50% of correlation coefficient (Tc) vs Hours

3. Conclusion

We proposed the use laser biospeckle to th study of microgravity effects on seed germination. Our preliminary results with the correlation of speckles show that this method can reveal the differences depending on the microgravity.

- Rajagopalan, U. M., Wakumoto, R., Endo, D., Hirai, M., Kono, T., Gonome, H., Kadono, H., & Yamada, J. (2021). Demonstration of laser biospeckle method for speedy in vivo evaluation of plant-sound interactions with arugula. *PLoS ONE*, 16(10), e0258973. <https://doi.org/10.1371/journal.pone.0258973>