Laser Speckle Imaging Reveals Bacterial Activity Within Colony

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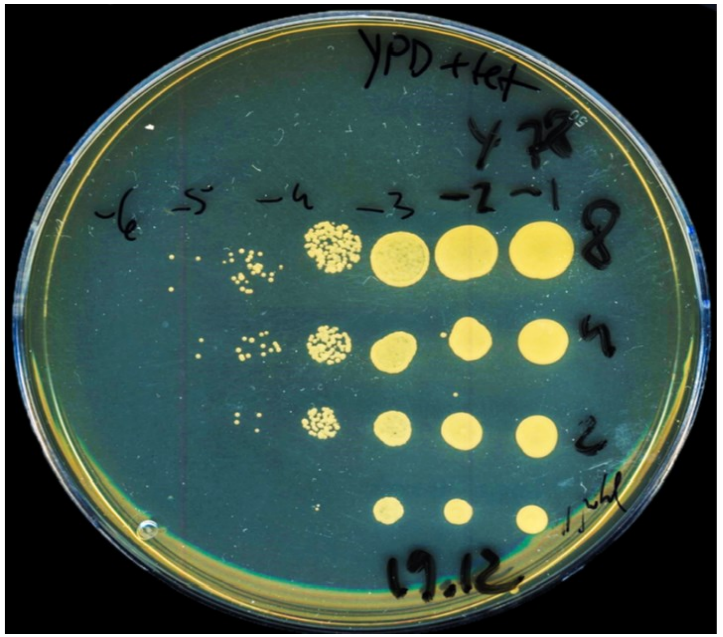
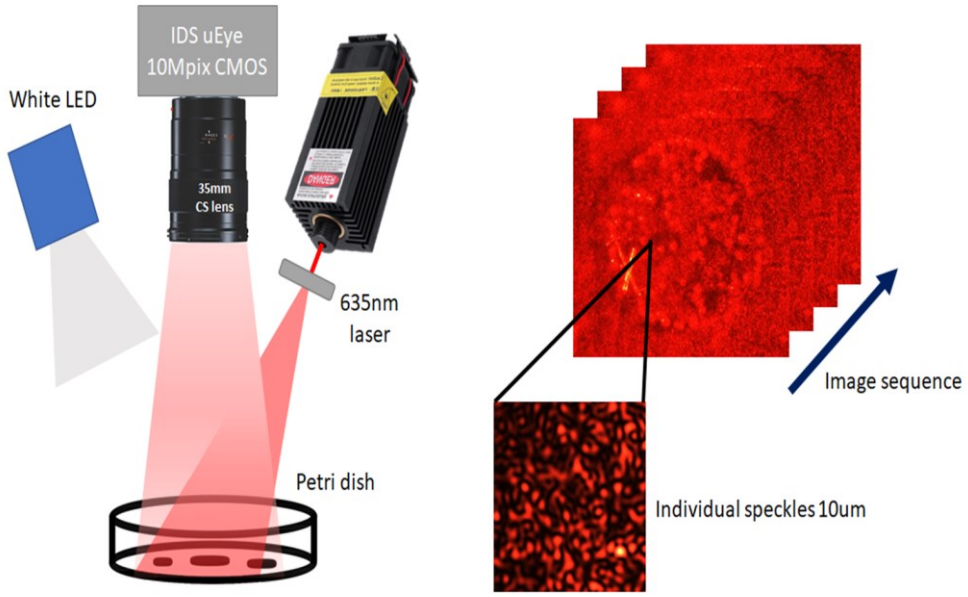
Introduction

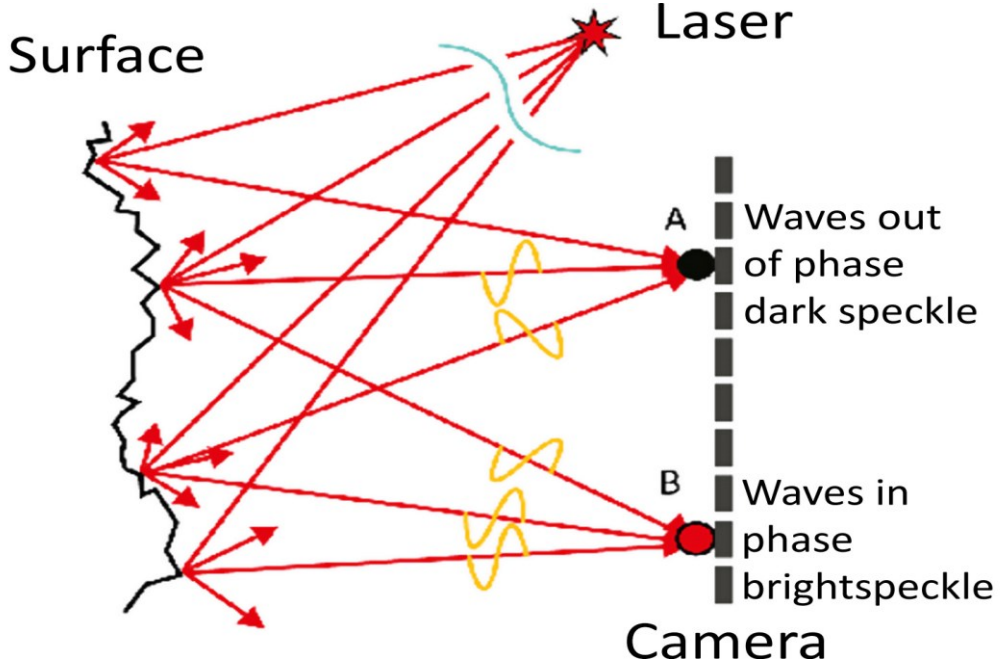
- In this study, a method is proposed to investigate bacterial activity on the colony edges. It is known that the peak of bacterial activity moves from the colony center to the edges.
- This phenomenon can be described using Pirt mathematical model for colony growth [1], where proposes that colony growth occurs due to the proliferation of a group of cells located on the edge of the colony. At the same time the center of the colony might become inactive [1].
- Currently no simple method exists how to visualize bacterial activity within the colony using non-invasive methods. Laser speckle imaging technique with increased sensitivity and signal processing algorithms based on correlation subpixel analysis can help to do this. This method can detect vibrations on the microbial colony surface due to bacterial activity [2,3].
- In this study, we show that this method can demonstrate differences in bacterial activity over time in the different parts of the colony.



The experimental setup and experiment description

- The laser speckles were generated by a 635 nm diode pumped solid state laser (output power 50 mW). Images were captured with 30-second intervals by CMOS camera.
- To study the changes of bacterial activity within a growing colony, we used Vibrio natriegens bacteria; we cultivated bacteria on solid, agarised media for 60-70 hours





Converting speckle image to time series (Algorithm description)

- 1.) Performing a two-dimensional normalized correlation between a fragment of the image allows you to find the changes in the speckle image that occur as a result of dynamic activity [4].
- 2.) Finding the location offset of the maximum correlation value determines the changes that occurred between frames.
- 3.) To find a more accurate value of the offset, interpolation was performed within the maximum of the correlation function [5].
- 4.) It is necessary to take into account previous offsets. Therefore, the offsets obtained between each pair of adjacent samples accumulate.

Converting speckle image to time series (Mathematical expressions)

$$1).Corr(u,v) = \frac{\sum_x \sum_y ((a(x,y)-\bar{a}) * (b(x-u,y-v)-\bar{b}))}{\sqrt{\sum_x \sum_y (a(x,y)-\bar{a})^2 * \sum_x \sum_y (b(x-u,y-v)-\bar{b})^2}}$$

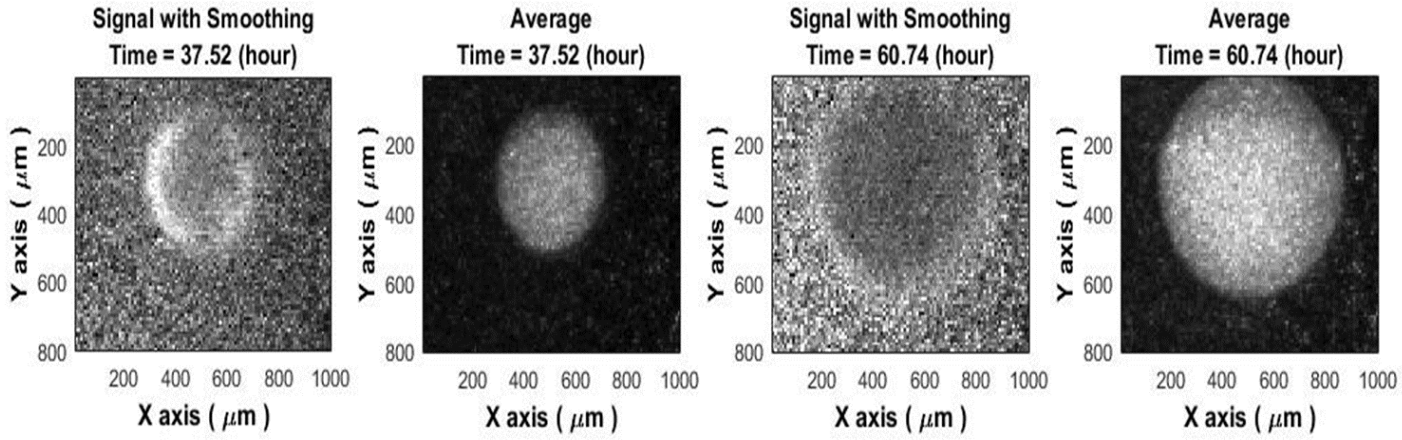
$$2).\left(\hat{u},\hat{v}\right) = \arg\max_{u,v}\left(Corr(u,v)\right)$$

$$3).\hat{\delta}_x = -\frac{b_u}{2a_u} = \frac{Corr(\hat{u}-1,\hat{v}) - Corr(\hat{u}+1,\hat{v})}{2\left(Corr(\hat{u}-1,\hat{v}) - 2Corr(\hat{u},\hat{v}) + Corr(\hat{u}+1,\hat{v})\right)}$$

$$4).sig[n] = \sum_{i=1}^{n} \hat{\delta}[i]$$

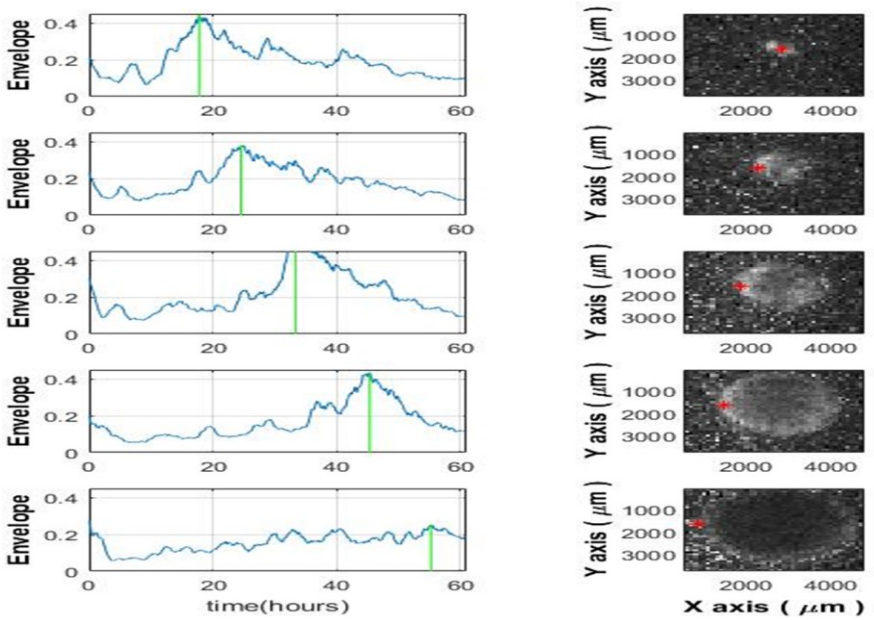
Algorithm Benefits.

1) It has been observed that by using averaging laser speckle imaging technique without additional processing, it is not possible to detect changes in activity at the edges of the colony. Whereas by sensitive correlation subpixel analysis, the changes in activity at the edges of the colony can be detected
2) The subpixel correlation technique allow to detect colonies earlier than using averaging.



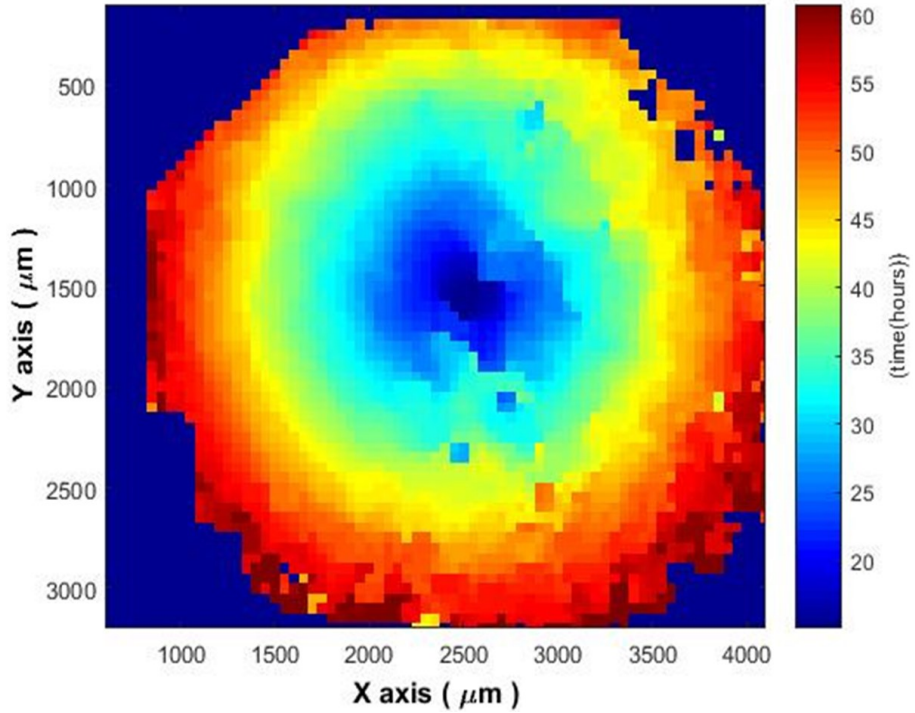
Results

Inside the colony the signal (which characterizes bacterial activity) increased, reached a maximum and then went down. The maximum value followed by corresponding decrease in activity at first were observed in the colony center. During the time activity decreases from the center toward the edges of the colony. In the center of the colony the maximum occurs earlier, at a distance from the center - later and at the latest at the edges of the colony.



Results

Having noted the times of the beginning of the decrease in activity throughout the entire area of the colony, it is possible to obtain a spatial map. Blue color represents the earliest, red color the latest. The "rings" are visible around the center and moving away indicating the decrease in activity in later times, with distance from the center.



Summary

1) The Pirt model describing growth of the colony is based on local nutrient depletion due colony growth [1]. The other authors have shown that due to restricted access to oxygen and depletion of nutrients right beneath the colony, the center of the colony is formed from dead cells mostly [6].
2) On current study we demonstrate that, indeed, this might be true proliferation activity within a colony follows a pattern of nutrient depletion (from center to edges).
3) The laser speckle imaging technique with increased sensitivity, based on sensitive correlation subpixel analysis, demonstrated that proliferation activity ceases over time from the center to the edges as the colony area increases.
4) We assume that this method would be helpful for applications in assays where bacterial activity is assayed [7].

References

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Thank you for your attention!