

Tracking Microbial Activity Using Laser Speckle Analysis

Ilze Lihacova^a, Valts Liepins^b, Eduards Teodors Mincis^c, Emilija Vija Plorina^{**a}, Edgars Kvisis-Kipge^a,
Alexey Lihachev^a, Janis Liepins^c

^aInstitute of Atomic Physics and Spectroscopy, Faculty of Science and Technology, University of Latvia, Riga, Latvia, LV-1004; ^bInstitute of Numerical Modelling, Faculty of Science and Technology, University of Latvia, Riga, Latvia, LV-1004; ^cInstitute of Microbiology and Biotechnology, Faculty of Medicine and Life Sciences, University of Latvia, Riga, Latvia, LV-1004
emilija_vija.plorina@lu.lv

Abstract: The laser speckle imaging system developed in this study monitored *Pleurotus ostreatus* growth in real-time. This non-invasive method reliably mapped microbial activity with low data volume and fast processing, accelerating microbiological experiments and enhancing high-throughput screening applications.

1. Introduction

Rapid and accurate microbial growth assessment is essential in medicine, food safety, and biotechnology. Traditional methods like optical density (OD), fluorescence assays, and colorimetric tests are commonly used but have limitations. For instance, OD measurements, while quick and simple, can be inaccurate if cultures form aggregates or biofilms that affect light transmission [1]. Moreover, OD cannot differentiate between live and dead cells, potentially leading to misleading results [2]. Fluorescence assays using dyes like resazurin or fluorescein diacetate assess microbial metabolic activity with greater sensitivity than OD measurements. However, they can be affected by sample autofluorescence or other fluorescent substances, leading to background noise and reduced precision [3].

Colorimetric tests detect microbial activity through color changes, such as tetrazolium salts converting to colored formazan by active cells. Although simpler, they are generally less sensitive and specific than fluorescence assays and can be influenced by sample color or turbidity [3].

These methods face limitations, including sensitivity and specificity issues, inability to distinguish living from dead cells, and interference from sample color, turbidity, or autofluorescence. Some require long incubation times or complex preparations, increasing cost and time, and are not always suitable for automation, limiting high-throughput applications.

Given these limitations, there is a need for novel, rapid, sensitive, and non-invasive methods to assess microbial growth. One promising approach is laser speckle imaging, which analyzes interference patterns generated by coherent light scattering. This technique enables real-time, non-contact evaluation of microbial activity, offering potentially higher sensitivity and specificity compared to traditional methods.

2. Equipment and methods

2.1. Sample preparation and experimental set up

In this study, *Pleurotus ostreatus* was used, which was cultivated in a Petri dish on a 2% barley extract agar medium for 6 days prior to the start of the measurements. A single-mode laser with a wavelength of 660 nm was employed to generate speckles and to illuminate the sample. The laser beam was uniformly diffused across the entire Petri dish to ensure even exposure. A 12.3 Mpix CMOS camera was used to capture images of microbial growth dynamics. Images were recorded over 70 hours, with 40 seconds of capture at the start of each hour at a frequency of 1 frame every 3 seconds. These measurements were conducted in an incubator at a temperature of 37°C.

2.2. Image processing methodology

Usually, imaging noise is undesired and considered an error of the measurement. In the case of this study the noise produced by laser speckle pattern is used to amplify miniscule changes in the region of interest. Considering that in this case noise is the carrier of information, precautions had to be made to avoid automatic noise reduction algorithms employed by the camera and image compression algorithms.

To extract the signal produced by speckle pattern dynamics the pixel intensities of each successive pair of speckle images were subtracted. By applying additional filters and thresholding each difference frame was binarized to differentiate pixels with and without significant change. The final processed image is produced by summing 16 successive binarized difference frames into one producing map of each pixel's dynamics. In which

case value of 0 shows that a pixel had no significant change over the span of 17 frames and value of 16 shows that a pixel had significant change in every captured frame.

3. Results

As a result, a series of RAW speckle images were obtained, clearly demonstrating the radial growth of the *Pleurotus ostreatus* colony from the center towards the edges (see Figure 1). In RAW laser speckle images, the location of the speckle colony is visible, but it is not possible to determine where microbial activity is occurring.

In the processed images (Figure 2), it can be observed that immediately after placing the sample under the speckle system, the activity of *Pleurotus ostreatus* is higher than during the rest of the experiment. We observed that the activity decreased until 13–14 hours and then gradually increased at the colony boundary.

Fig. 1 RAW laser speckle images of *Pleurotus ostreatus* colony captured at different growth stages: at the start of the measurement (0h) and during growth at 14h, 28h, 42h, 56h, and 71h.

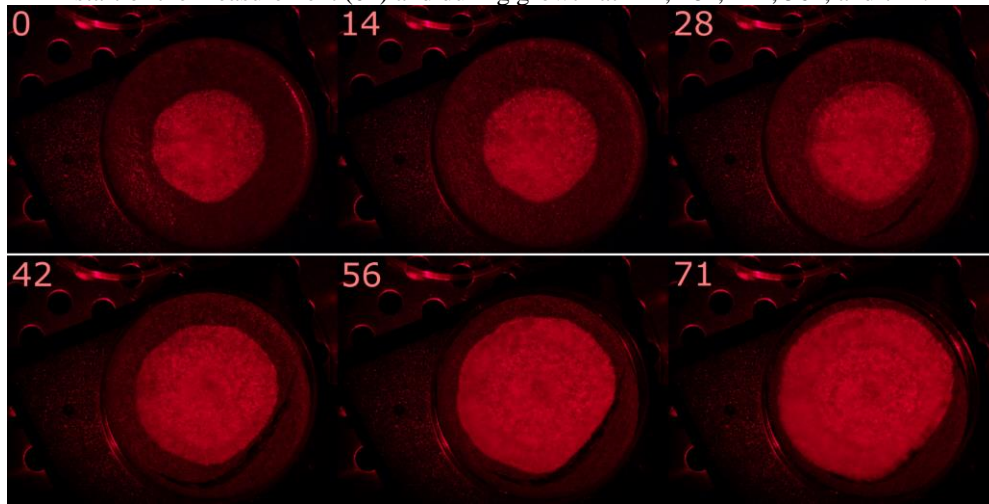
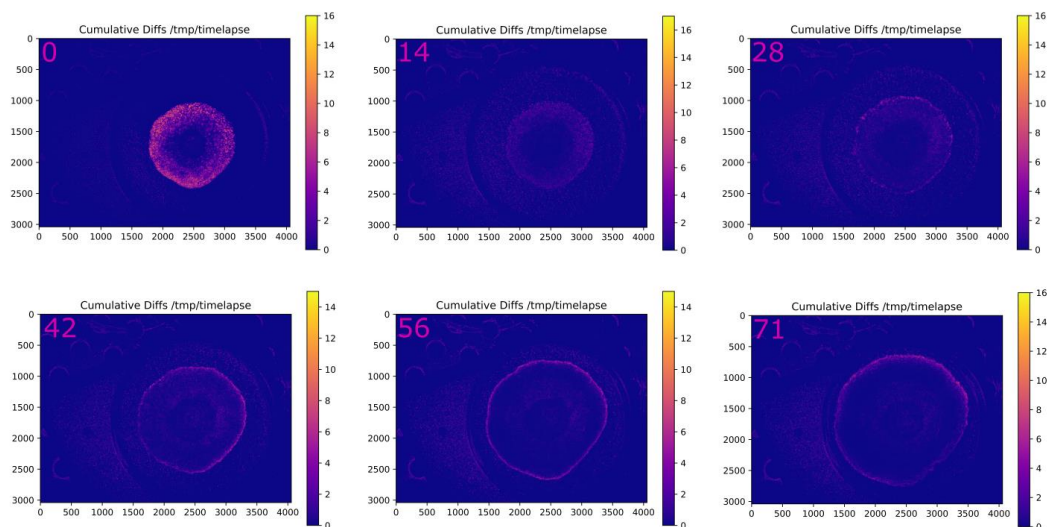


Fig. 2. Processed laser speckle images of *Pleurotus ostreatus* colony showing activity intensity at different growth stages: at the start of the measurement (0h) and during growth at 14h, 28h, 42h, 56h, and 71h.



4. Discussion

In our experiment, we observed that microbial activity during growth was concentrated at the colony's periphery. This observation aligns with the Pirt model, which describes how nutrient and oxygen gradients within a microbial colony lead to increased metabolic activity at the edges, where resources are more abundant [4]. Similar results were

obtained using the cross-correlation processing method, which has been developed in this field by I. Balmages *et al* [5].

The main advantage of the method described in this study is its short processing time, small data volume, and reliable results, enabling real-time monitoring of microbial activity at any point during the experiment. This approach is particularly useful in experiments where microbial activity is not directly visible in RAW images.

Potential applications include studying biofilm formation, monitoring the growth dynamics of fungal or bacterial colonies, and assessing antimicrobial efficacy. Additionally, it can be used in food safety testing to detect microbial contamination or in environmental monitoring to observe microbial activity in soil or water samples.

5. Conclusions

In conclusion, our research group has successfully developed a method capable of real-time monitoring of microbial activity. This approach provides reliable results with short processing times and low data volume, making it suitable for experiments where microbial activity is not directly visible in RAW images.

Future studies will focus on adapting this method for use with multi-well plates, enabling simultaneous monitoring of multiple experiments in laboratory settings. This advancement could significantly enhance high-throughput screening in microbiological research.

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