

# TRACKING MICROBIAL ACTIVITY USING LASER SPECKLE ANALYSIS

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## ABSTRACT

Rapid microbial activity assessment is vital in medicine, food safety, and biotechnology. This study demonstrates laser speckle imaging as a non-invasive, real-time method for monitoring growth and activity profile of oyster mushroom, *Pleurotus ostreatus*. A 660 nm laser and CMOS camera captured speckle patterns over 70 hours. Image processing techniques enhanced

visualization, revealing activity concentrated at the colony edge, consistent with nutrient and oxygen gradients. The method provides low data volume, fast processing, and accurate detection without disturbing samples, making it suitable for applications in biofilm research, antimicrobial testing, and environmental monitoring.

## EQUIPMENT AND METHODS

### Sample Preparation and Experimental Setup

*P. ostreatus* was cultivated on 2% malt extract agar in a Petri dish for six days before measurements. A single-mode 660 nm laser was used to illuminate the sample, with the beam evenly diffused across the entire dish (Fig. 2.). A 12.3 MP CMOS camera captured speckle images over 70 hours, recording 40-second sequences every hour at a rate of one frame every 3 seconds. All measurements were conducted at 37°C in an incubator.

### Image Processing Methodology

In this study, laser speckle noise was used as a source of information, amplifying subtle changes in microbial activity. To preserve meaningful speckle variations, automatic camera noise reduction and compression were avoided. Image sequences were processed by subtracting pixel intensities of successive frames. These difference images were binarized through filtering and thresholding to highlight significant changes. Sixteen consecutive binarized frames were then summed to generate a dynamic activity map, where a pixel value of 0 indicates no significant change, and 16 indicates consistent change in every frame.



Fig. 2. Experimental setup comprising a 660 nm single-mode laser for uniform sample illumination and a 12.3 MP CMOS camera for capturing microbial growth dynamics.

## 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.

## INTRODUCTION

Accurate microbial growth assessment is crucial in medicine, food safety, and biotechnology. Traditional methods like optical density [1], fluorescence assays [2], and colorimetric tests [3] often lack sensitivity, specificity, or the ability to distinguish live from dead cells. They may also be affected by sample turbidity, color, or autofluorescence, and often require lengthy preparation or incubation.

These limitations hinder automation and high-throughput use. Laser speckle imaging offers a promising alternative by analyzing interference patterns from scattered coherent light, enabling rapid, non-invasive, and real-time monitoring of microbial activity (Fig. 1.) with potentially greater sensitivity and specificity than conventional techniques.

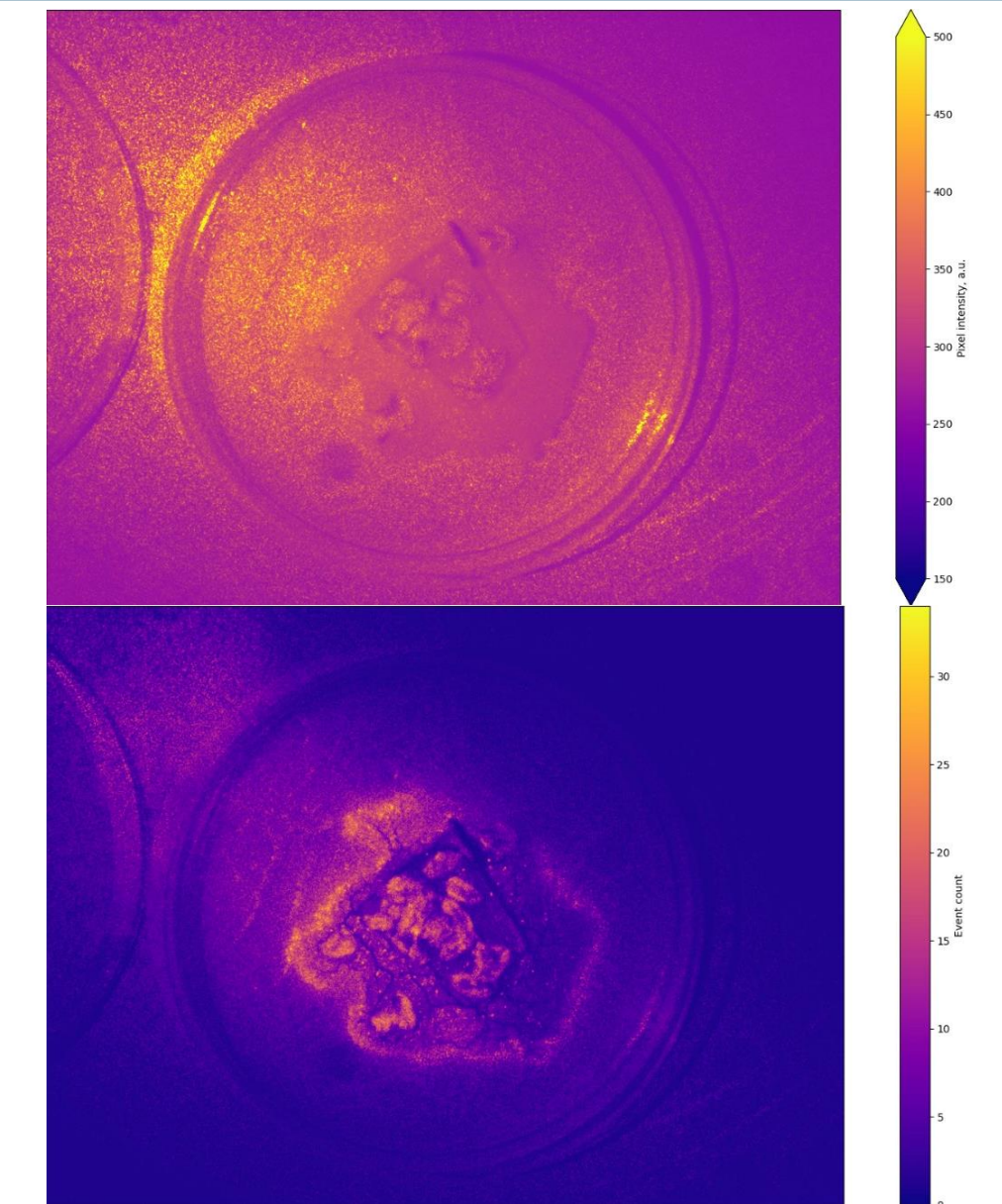


Fig. 1. Unprocessed image (top) of the slime mould (*Physarum polycephalum*) and the corresponding activity map (bottom) obtained through speckle pattern analysis, highlighting active regions.

## RESULTS

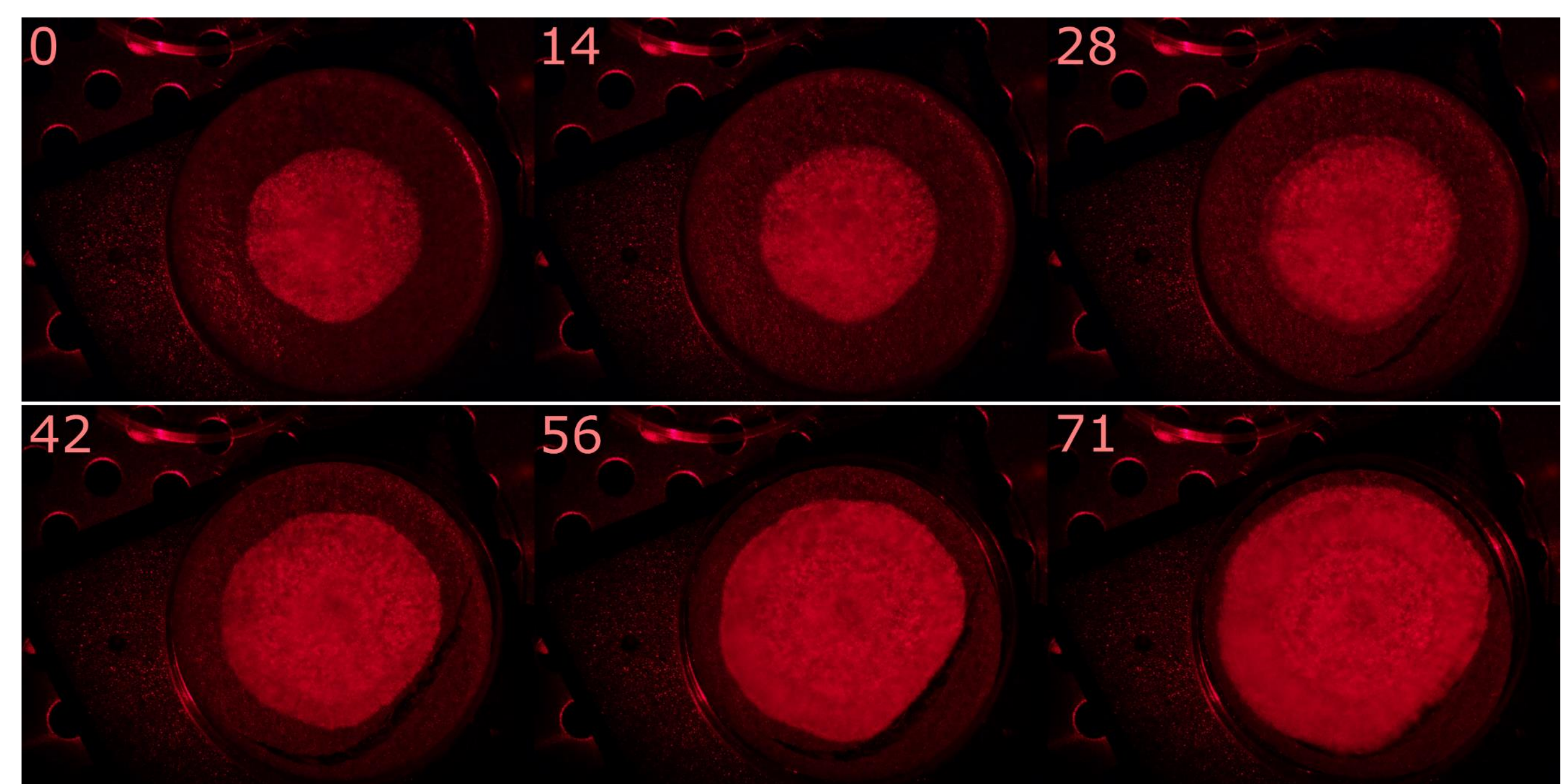


Fig. 3. RAW laser speckle images of *Pleurotus ostreatus* colony captured at different growth stages: at the start of the measurement (0h) and during growth at 14h, 28h, 42h, 56h, and 71h.

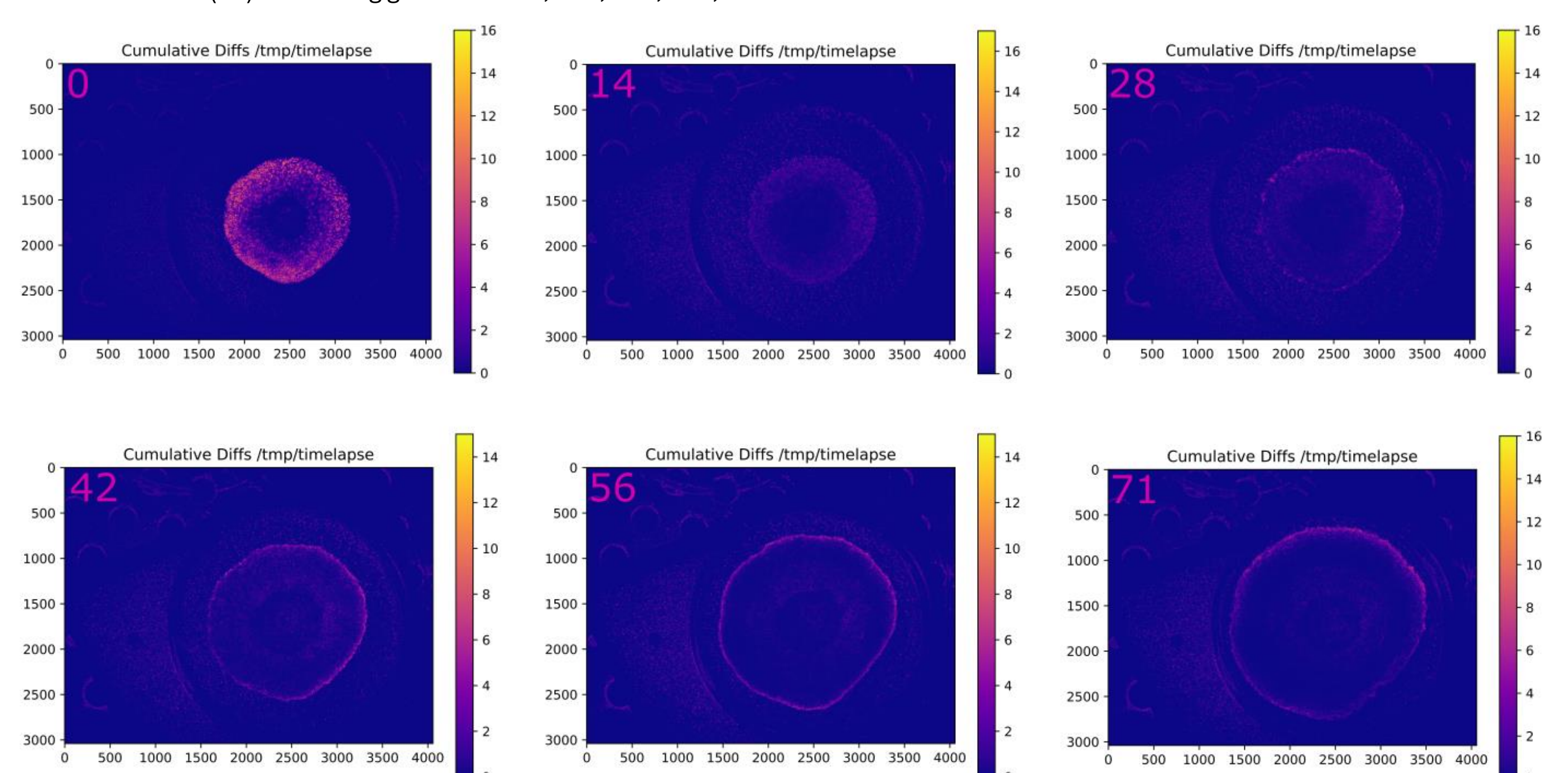


Fig. 4. Processed laser speckle images of *Pleurotus ostreatus* colony showing activity intensity at different growth stages: at the start of the measurement (0h) and during growth at 14h, 28h, 42h, 56h, and 71h.

As a result, a series of RAW speckle images were obtained, clearly demonstrating the radial growth of the *P. ostreatus* colony from the center towards the edges (Fig. 3). In RAW laser speckle images, the total colony is visible, but it is not clear if all of the colony is active or not.

In the processed images (Fig. 4), it can be observed that immediately after the sample is placed under the speckle system, the activity zone of *P. ostreatus* is more pronounced than during the rest of the experiment. Potentially this is

related to substrate consumption pattern of the microbial colonies.

The RAW speckle images do not reflect the actual activity zones within the colony — it is unclear if the colony is actively growing, and whether metabolic activity occurs throughout the entire colony or only at the edges (as observed between 56 and 71 hours).

## 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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