



RESEARCH ARTICLE

Bio-Speckle Laser Technology In Revealing Microbial Radial Growth: A Review

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ARTICLE INFO	ABSTRACT
Received: Apr 24, 2024 Accepted: June 13, 2024	In culture, observation of the microorganism development is significant in microbiology for the growth kinetics research and optimization, biomass, and metabolite synthesis. The technique of Biospeckle laser technology is non-invasive facilitating the monitoring regions with increased microbial activity in the colony. Effectiveness of Biospeckle laser technology (BLT) appeared in several research works, as a reliable approach for the observations of the kinetics of bacterial growth. In these works, the bacterial proliferation reduces both speckle grain sizes and spatial contrasts. Also, the speckle parameters show a high sensitivity degree to the fermentation conditions. This work examines speckle patterns and mathematical techniques for assessing the biospeckle activities, and the use of these techniques for showing microbial radial growth.
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INTRODUCTION

Microbial colonies growth in a radial manner is complex influenced by many things, important in the estimating the colony appearance time which is colony's radial growth rate (GR). The environmental conditions could affect the way the microorganism grows in radial patterns. For instance, the microorganism growth in biofilms is a dynamic ecosystem on many surfaces. In newly formed bacteria microorganisms grow in radial patterns and physically moved from the center of the colony growing outward in radial or circular ways. Many elements such as the concentration of nutrients, environmental conditions, and culture parameters affect the growth. For instance, bacteria spread more quickly if a higher nutrient concentration appears which cause a more fractal patterns at lower and compact growth patterns with the rise in the nutrient density (Del Giorgio et al., 2011). Also, environmental signals are pivotal in the growth and dispersal of microorganisms in biofilms, these signals affect how microorganisms grow in radial patterns (Allen & Waclaw, 2018). bacterial growth is influenced by Several growth factors, which can be categorized into environmental factors including, Oxygen levels, pH levels, Moisture, Temperature, and Nutritional factors. Bacteria move radially from the center toward the periphery of the colony as a result of cell growth in accordance with some factors including, bacterial secretions, nutrient availability, cell growth,

division of cells, and mechanical forces (Rasul et al., 2023). Bacteria exist in natural habitats and the surrounding environment. The majority of microorganisms perform vital functions in the natural environment, and numerous species have mutually beneficial relationships with plants or animals (Weiland-Bräuer, 2021). These renowned microorganisms are the initial illustration of the biotechnological procedure, occurring via the fermentation of various substances, such as milk, meat, fish, grapes, and grains, to generate essential food items for human consumption. The utilization of these microorganisms is crucial for the creation of novel functional products or ingredients that include, for example, probiotic/synbiotic formulas that are trapped within secure matrices. Furthermore, in this particular state, their vitality remains intact during certain food processing methods or throughout the digestive system (Oleandro et al., 2021).

Concurrently, many bacteria are undesirable or even pathogenic, contributing in certain cases to modify the quality and beneficial attributes of food, and in numerous instances to induce significant ailments (in humans, animals, or even plants) (Nath et al., 2022). Typically, they can be found in various settings, including the microbiota of both animals and plants, as well as in water contaminated with fecal matter (Rath, 2021).

Microbial infections are a leading cause of mortality in numerous underdeveloped nations worldwide (Salam et al., 2023). These bacteria exhibit resilience to environmental circumstances. Furthermore, the vast majority of the human population is completely vulnerable, and the illnesses they induce are severe, often resulting in a significant number of deaths. A substantial quantity of these deadly bacteria can easily be cultivated and preserved for extended periods of time. Furthermore, there is a growing prevalence of pathogens that have developed resistance to traditional pharmaceuticals. Typically, bacteria exist in the planktonic state. As the quantity of microbial cells grows, they start to form organized microbial communities, surrounded by a substance called biofilms. These biofilms can adhere to any surface, including animals, plants, minerals, sediments, soil, and various biomedical implants and transcutaneous devices (Barrantes et al., 2021).

The physiological characteristics of bacteria during biofilm formation differ from those in the planktonic state. In this modified state, they exhibit increased resistance to antibiotics and other biocides due to the protective envelope created by exopolysaccharides and proteins (Conlon et al., 2013). Hence, it is crucial for both the health and food industry to have advanced methodologies that are highly sensitive and capable of rapidly detecting and monitoring microbial populations in a specific biological matrix. These methodologies should also enable the study of all metabolic changes, substituted by bio-molecular technologies in the field of critical microbiology (Kumar et al., 2022). However, while these approaches are indeed quicker than the previous ones, they necessitate specific initial procedures (such as DNA extraction, gene amplification using PCR-RT, and material analysis), which might still result in an undesirable waste of time (Maurer, 2011). For biofilms, traditional techniques that rely on plating and microscopic counting are appropriate for investigating and identifying the quantity of bacteria that attach to eukaryotic cells or non-living surfaces (Bhunia et al., 2022).

Colony growth, heat evolution, oxygen consumption rate, motility, and cell nucleic acid concentration are some of the criteria used to examine microorganisms. Microorganisms, when introduced to a medium and kept in optimal conditions, exhibit various physical and chemical alterations in it as a result of their metabolic, reproductive, migratory, and other activities. When there is a significant increase in the number of microbes as a result of multiplication, they have the ability to spread over solid surfaces through a process known as sliding (Omae et al., 2012).

The formation of bacterial colonies has been studied using image approaches which extensively utilize low coherent light (Moon & Javidi, 2008). By using light with a long coherence length instead of low coherent light, grainy looking images are produced. This is caused by a speckle pattern which is a random interference pattern (Moon & Javidi, 2008). When the amplitude distribution and phase of the light in the biological medium vary over time, changes also occur to the structure of the speckle pattern. "dynamic speckle" or

"biospeckle" patterns is the term used to describe these kinds of speckled structures that change over time (Jam et al., 2018; Rabal & Braga Jr, 2018).

The optical characteristics of the medium undergo changes in accordance to microbe activity, establishing a relationship between the dynamics of speckle pattern activity and microorganism activity. A benefit of dynamic speckle techniques for assessing microorganism activity is the operation procedures, the simplicity of the optical equipment, and the non-contact regime. This technique is non-invasive and can be used to assess biological processes in which invasive measures are not needed, unlike previous techniques (Aronsson & Rönner, 2001; Minz & Nirala, 2014; Ramírez et al., 2009).

The quantification the living microorganisms in a sample is a key target of epidemiology. Every living cell triggers the formation of a single colony on an agar plate medium known as colony forming units (CFU). After they become readily discernible to the untrained eye the colonies are enumerated. A waiting period of 18-40 hours is typically needed for the CFU's to mature before they can be counted in conventional techniques. The quantification of viable microbial cells is important in fields such as veterinary industry, pharmacy, medical, food production, and environmental observation, as it impacts directly the health concerns of patients and society (Da Silva et al., 2018).

The Accurate determination the exact quantity of living microorganisms in a sample is crucial for obtaining prompt findings. Therefore, the early identification of microbe proliferation is enabled through the implementation of novel techniques, beyond the capabilities of current approaches, which leads to the enhancement of the effectiveness of epidemiological efforts (Rohde et al., 2017).

1. MODELING OF MICROBIAL COLONY GROWTH

The radial growth of microorganisms is a complex phenomenon influenced by myriad of factors, understanding these complexities is a key factor for the development of effective techniques to analyze, observe, and interpret radial growth patterns. Among the first models that described microbial colony radial growth over time is the Pirt model of microbial colony growth. The model aligns with real observations if no growth inhibition is shown by the adjacent colonies and the size of the activity zone on the edge is stable (Peleg & Corradini, 2011). A commonly used sigmoid model that describes the growth of microorganisms, which includes fungi and bacteria is the Gompertz model of microbial colony growth as time passes. It has been used on various growth data and it was a great fit with experimental observations. The Gompertz model is associated with Richards family of three-parameter sigmoidal growth models and is a unique case of the four-parameter Richards model (Allen & Waclaw, 2018). This model was used to describe the microbial growth in different contexts, including:

The growth of bacteria in food products (Balmages et al., 2023; Rashid et al., 2023; Kanval et al., 2024). Yeast colonies growth on agar medium. The growth of bacteria in mixed cultures (Tonner et al., 2020). The advantages of this model include, for example different growth conditions adaptability, simplicity, and growth kinetics quantifying ability. nevertheless, it is best to keep in mind that this model may not always be the best fit for all growth data, and alternative models like the Baranyi model or logistic models may be more appropriate in a few cases (Cao et al., 2020). The Gompertz model describes microbial colony growth as time passes as commonly utilized providing a simple framework for the prediction and understanding of the growth kinetics in various contexts:

$$N(t) = N_0 e^{-e^{\frac{GR}{N_0}(t-T_{lag})+1}} \quad (1)$$

(N_0) is the initial number of cells, ($N(t)$) the number of cells at time t , (T_{lag}) the lag time, (GR) the growth rate, and (e) is the base of the natural logarithm (Rivas et al., 2014). In the

Gompertz equation, the colony approaches its carrying capacity of the growth rate of the colony which reduces exponentially. The model is based on the idea that the growth rate is in proportion to the number of cells in the colony, and the finite colony's carrying capacity. The model is adaptable to numerous growth conditions after applying to various growth data which provide a framework to understand and predict growth kinetics in many contexts (Rivas et al., 2014). To estimate growth parameters such as the maximum growth rate, lag time, and carrying capacity, this equation describes microbial growth through fitting the equation to the experimental data. This is in various contexts: growth of yeast colonies on agar medium, of bacteria in food products, and in mixed cultures (Peleg & Corradini, 2011; Rivas et al., 2014).

This review focuses on the applications, instruments, and Biospeckle Laser principles to characterize microbial growth. Authors provide a brief overview of important research that use to examine the production of biofilms has been examined by the use of Biospeckle patterns with the exploration of the expansion of fungal mycelium, of bacterial colonies, and the behaviors of microbial movement. This work also overviews the existing difficulties and limitations. It was concluded that the analysis showcases the considerable capacity of Biospeckle patterns as a non-intrusive, label-free method for understanding the processes of radial microbial growth.

2. BIOSPECKLE TECHNOLOGY

Biospeckle technology is a novel tool for applications in food quality, food safety, and nutraceuticals. This technology enables rapid evaluation and monitoring of the presence of bacteria or their growth in a solid or liquid biological matrix. In addition, by reducing the connection between biospeckle patterns, authors can accurately measure and improve the duration of storage for probiotic bacteria enclosed in alginate, as well as their survival rate under simulated gastrointestinal circumstances (Pandiselvam et al., 2020).

For stationary, non-moving objects that scatter light, the resulting dispersed light forms consistent patterns of laser speckles. Nevertheless, when dispersed entities such as particles inside a fluid exhibit autonomous motion, such as Brownian motion, the individual speckle appears to exhibit a visual effect resembling rapid and irregular changes in brightness, sometimes described as "twinkling" or "boiling". The term used to describe this phenomenon is "time-varying speckle." Concerning this occurrence, the Biospeckle technique has been validated for monitoring the movement of particles in optically heterogeneous environments by the analysis of time-varying laser speckle patterns (Balmages et al., 2021).

Consequently, analyzing laser speckle patterns offers a potent means of measuring movement activity at both the micro and macroscale. Correlation approaches are currently a common choice for analyzing time-variable laser speckle patterns. If a rough surface is deformed, shook, or displaced, the resulting offset or displacement can be noticed in the speckle patterns (Gåsvik, 2003). The offset can be determined by the location of the peak in the cross-correlation function between frames. The autocorrelation peak's offset, when compared to the frame itself, is consistently zero. A cross-correlation peak offset (between frames) suggests the presence of a bias between them. The correlation coefficient is a useful tool for analyzing the activity of a time-varying speckle pattern (Federico et al., 2006). Every frame in the sequence is compared to the frame that came before it. Therefore, a variation in the correlation coefficient is obtained as time progresses. The limited temporal fluctuation of the correlation coefficient in the studied area indicates that it is relatively inactive within the sensitivity range of the detection method. The correlation coefficient graph, plotted against the frame number or time, represents the activity of the observed process (Federico et al., 2006).

Existing literature demonstrates notable progress in biospeckle laser techniques and their promise for assessing dynamic processes in microbiological medium. Laser speckle-based methods can be employed to assess the chemotactic response of bacteria in agar plates (Murialdo et al., 2009), as well as to differentiate motile bacteria from fungi (Murialdo et al., 2012). The use of speckle decorrelation time maps has been shown to detect *Escherichia coli* (*E. coli*) and *Bacillus cereus* on chicken breast meat (Yoon et al., 2016).

Speckle analysis has also been used for estimating the kinetics of biomass growth in liquid culture (Loutfi et al., 2020b), examining the morphology of CFUs (Kim et al., 2014), and determining antibiotic susceptibility (Grassi et al., 2016). A rapid system response in evaluating antibiotic susceptibility in minimum inhibitory concentration testing has shown importance of laser speckle techniques, along with Deep Learning and Artificial Neural Networks (Zhou et al., 2020). Overall, the Biospeckle laser approach is straightforward, offering prompt detection of microbiological activity compared to methods relying on turbidity estimation or manual colony counting.

3. BIOSPECKLE ANALYSIS

Mathematically speaking, speckle can be seen as a random movement in the complex plane (Goodman, 1976). When a beam of light with a consistent wavelength interacts with uneven surfaces, it creates patterns of light and dark spots on the equipment used to capture the image. If the roughness of the surface is similar to the wavelength of the probe, we may represent the surface being tested as a collection of L separate scatterers. These scatterers can be thought of as L distinct point sources located at different 3D coordinates. The detector receives light that has been dispersed from each location and has traveled through a medium such as air, PBS, or water, resulting in a varied optical path. Hence, the detector receives the combined and synchronized L complex wave front contributions, resulting in an intensity pattern.

$$I = \left| \sum_{i=1}^L U_i \right|^2 = \left| \sum_{i=1}^L P_i e^{f\varphi_i} \right|^2 = \left| \sum_{i=1}^L P_i e^{f\psi_i} \right|^2 = \left| \sum_{i=1}^L P_i e^{f \frac{2\pi}{\lambda} OPD_i} \right|^2 \quad (2)$$

where $U_i \in \mathbf{C2}$ denotes each single scattering contribution; ρ_i and ψ_i represent the modulus and period of the i -th term; OPD_i represents the corresponding experienced optical path delay; and λ is the wavelength (the dependence on the spatial variables has been excluded in Equation (1) for the sake of conciseness).

Speckle is commonly seen as a hindrance to imaging, and numerous approaches for reducing speckle have been suggested throughout the years. Speckle grains are a valuable source of information as they provide a coherent sum that is sensitive to small time fluctuations in the rough surface, namely changes that occur on the scale of the probe wavelength. When studying the creation of speckle patterns in microscope settings, it is advantageous to analyze the patterns that occur when light is reflected or refracted from a scattering medium. This analysis provides valuable information about the medium itself. Moreover, the dimensions, arrangement in space, and occurrence pattern of the speckle particles can be associated with the form of the item causing the scattering, its level of unevenness, and the density on its surface or within its volume (Bianco et al., 2016, 2018). Due to its high sensitivity, optical speckle metrology has become widely used in several industrial sectors for non-disruptive testing of large items (Kreis, 2006).

Within the realm of techniques that utilize speckles to gather information about biological activity within a given field of view (FoV), two main categories can be identified: static approaches, in which speckles remain unchanged over time, and dynamic approaches, which are affected by the optical Doppler effect and result in speckle variations over time. Hence, the fluctuating speckles encompass data regarding the movement of the object or the mobility of particles within the item (Oleandro et al., 2021).

BLT has numerous benefits that make it extremely appropriate for studying the dynamics and behaviors of microbial growth: BLT enables the capture of colony growth and movement with high precision and speed using quick wide-field imaging, providing remarkable spatiotemporal resolution, BLT has been widely used to analyze the spatiotemporal growth patterns of microbial colonies and extract important phenotypic data (Ansari & Mujeeb, 2018). Ansari et al. employed BLT in their investigation to examine the production of biofilms and the movement of bacteria with a time resolution of less than one second. This experiment revealed the existence of intricate dendritic structures with fractal-like patterns and fast

movement on the surface. BLT enables the imaging of microbiological samples over long periods of time without causing any disturbance, thanks to its non-contact nature (van der Kooij, 2020).

Van der Kooij et al. observed the growth and maturation of biofilms by performing uninterrupted BLT measurements minimum sample preparation compared to techniques that depend on exogenous markers or stains. The lack of physical obstruction enabled the examination of prolonged quantitative expansion Van der Kooij et al. demonstrated that BLT has the ability to reveal information on the diversity of individuals within a population and the three-dimensional structure of biofilm clusters by analyzing temporal speckle statistics (van der Kooij, 2020).

The radial expansion rates assessed by BLT are highly consistent with the growth curves obtained using conventional approaches such as optical density and colony counting (Rohde et al., 2017). The work conducted by Nixon-Luke et al. employed the use of BLT to observe the radial expansion of *Staphylococcus aureus* colonies on agar with radial expansion curves obtained via BLT closely matched the OD600 growth curves, confirming the reliability of BLT for quantitative growth analysis.

Uninterrupted, extended time-lapse imaging to study the evolving microbial growth patterns caused by genetic variables and various environmental conditions is possible by the use of BLT (Rohde et al., 2017). Kasimov et al. conducted a prolonged BLT surveillance of the growth of *E. coli* colonies over a range of temperatures, spanning from 20°C to 37°C. The BLT growth curves show the influence of temperature on the lag time, exponential growth rate, and carrying capacity in a manner that was reliant on it.

Accurate analysis of morphological development, such as fractal-like branching patterns and filamentous fungal differentiation, in addition to radial dynamics is enabled by BLT (Pandiselvam et al., 2020). Buchberger et al. represented the filamentous architecture and development dynamics of *Neurospora crassa* using BLT. Insights into the intricate structure and growth patterns of the branching network and hyphal movement were yielded by the use of BLT. Moreover, the examination of dynamic speckle variations yields distinct patterns of growth that are unique to particular bacterial species and strains (Aronsson & Rönner, 2001). Ansari et al. demonstrated that BLT has the ability to distinguish between *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* by utilizing distinct temporal and spatial texture characteristics.

Specifically, light-scattering issue use a hemisphere-capped cylinder model to determine the length and diameter of individual *E. coli* bacteria, with each element being shaped accordingly (Ansari & Mujeeb, 2018; van der Kooij, 2020). The classification of four different bacteria species was facilitated by the rod-like structure when analyzing the Fourier transform light scattering pattern (Jo et al., 2015).

Hence, it is crucial to develop rapid detection, classification, and discriminating methods for foodborne bacterial pathogens that exhibit high sensitivity and specificity. These systems play a critical role in food safety control and help prevent the spread of harmful pathogens in the food supply. This topic is extensively discussed in reference (Liu & Ngadi, 2013).

Instead of examining the stationary characteristics of scattering patterns, the methods that rely on the temporal fluctuations of the biospeckles investigate the temporal decorrelation that arises due to biological activity inside the FoV (Ansari et al., 2016; Bianco et al., 2017; Mandracchia et al., 2018). In this form of analysis, one can examine the function $U_i(t)$. Furthermore, it can be expressed as $I = I(x,y;t)$, with x and y representing the geographical variables and t representing the time variable. The presence of any small irregularities on the surface of the item, even if they cannot be seen with the naked eye, causes a change in the combined effect described by Equation (1). This leads to variations in the intensity and positions of the speckles, which can be observed. The efficacy of this form of analysis primarily stems from the uncomplicated nature of the recording arrangement. To analyze and record the scattered speckle pattern for a specified time window, one only needs to shine coherent light over the surface (Ansari et al., 2016; Bianco et al., 2017; Mandracchia et al., 2018).

The utilization of the dynamic speckle approach in conjunction with temporal difference processing showed a comprehensive characterization of *E. coli*. The study observed the activity of coli bacteria in Petri dishes, distinguishing between the compartment that was infected with bacteria and the compartment that was not, at various phases of bacterial growth (Ramírez-Miquet et al., 2012). However, the potential of biospeckle pictures in assessing bacterial chemotactic response (Murialdo et al., 2009) and differentiating the activity profile of bacterial cultures has been tested (Ramírez-Miquet et al., 2013). The dynamic laser speckle method has proven to be more effective than traditional techniques in distinguishing between filamentous fungi and motile bacteria on agar plates (Murialdo et al., 2012). The reduction in speckle particle size resulting from an increase in the medium scattering coefficient has facilitated the identification of two distinct bacterial growth phases: an exponential phase characterized by bacterial proliferation, and a stationary phase suggesting sporulation and cell lysis (Loutfi et al., 2020a).

as well as to identify the presence of *E. coli*. The detection of *E. coli* and *Bacillus cereus* (Yoon et al., 2016) can be used to differentiate between fresh and contaminated water (Bianco et al., 2017). The optical lab-limited approaches, such as the dynamic laser speckle (DLS) methods, have been previously implemented (Braga, 2017; Catalano et al., 2019).

Furthermore, the recent progress in machine learning has propelled the expansion of AI-assisted biospeckle technique. The use of multiple descriptors can categorize diverse bioactivities that occur in different parts of the specimen surface that are not next to each other (Passoni et al., 2013). A notable instance of this is the ability to digitally identify and differentiate healthy and bruised areas in apples using this approach (Zhang et al., 2018).

The phenomenon of self-propelling bacteria is of significant significance as it causes a bacterial colony to disrupt the patterns of speckles over time, acting as a moving diffuser (Bianco et al., 2015). Simply put, the bacteria migrate over distances higher than λ , thereby altering the perception of surface roughness by the probing light.

Kasimov et al. (2020) used BLT to contrast the developmental patterns of *Bacillus subtilis* strains with intact genetic structure against mutants that doesn't contain genes responsible for motility and matrix production. Valuable information regarding the mechanobiology of colonies was yielded by examining the variations in radial velocities and morphological characteristics (Bianco et al., 2018).

(Briers et al., 2013) Observed the development of bacterial colonies and determined their radial growth rates by conducting a lengthened BLT study. BLT was used to demonstrate the

speckle variation rates, after that they were then processed to create profiles of radial velocity. The usual microbial growth curves obtained using plate counting and optical density methods closely resembled the radial growth rates calculated by BLT, for both Gram-negative and Gram-positive species. This experiment emphasized the precision of BLT in quantitatively assessing microbial growth factors.

(Pérez-González et al., 2015) Recounted the structural progression of bacterial colonies using BLT. Valuable information on the maturation processes was yielded using the quantification of motility behaviors and extracellular matrix production. The examination of fractal-like branching structures in *Bacillus subtilis* swarming and colony formation was facilitated by BLT. These results demonstrate the usefulness of BLT in understanding intricate microbial developmental adaptations.

(Ansari et al., 2018) Measured the movement patterns of bacteria at different concentrations in order to investigate the dynamics of quorum sensing using BLT. Autoinducer-2 signaling was linked to both increases in overall activity and local alignment movements. Therefore, BLT can clarify the behavioral reactions that underlie the mechanisms of cell-cell microbial communication.

Authors of another study employed an BLT -based method to facilitate the simultaneous monitoring of growth characteristics in a variety of bacterial isolates. Through the process of measuring temporal speckle fluctuations originating from each colony location, researchers were able to automatically derive isolate-specific measurements of growth rate and shape. This demonstrates how BLT enables quick measurement of phenotypic diversity in extensive bacterial datasets (Balmages et al., 2021).

In a different study, (Zhou et al., 2020) utilized BLT to perform parallelized antibiotic susceptibility testing for several bacterial strains and medications. They evaluated the rate of speckle fluctuation changes after administering the medicine to measure how it kills cells in a dose-dependent manner. From this, they determined the minimal inhibitory concentration values. This showcased the capacity of BLT to automate the susceptibility profiling required for high-throughput antimicrobial screening and precision medicine applications.

In the future, more improvements could increase the efficiency of BLT based screening, allowing it to handle even larger volumes of data, which would be acceptable for industrial and clinical applications. Continuous processing could be facilitated by the development of microfluidic integration and automated sampling techniques.

The implemented digital image processing technique has proven to be appropriate for monitoring the movement and structural alterations in the bacterial population over a period of time, as well as for identifying and differentiating the immediate effects of drugs on parasites.

4. CONCLUSION

We consider BLT method to be highly effective for rapidly monitoring bacterial development and promptly assessing the ability of bacteria to form a biofilm, even in the presence of a small bacterial load. Consequently, BLT method would significantly impact the timely identification

of bacteria in many types of substances, particularly in food and healthcare settings, enabling a rapid assessment of antibiotic resistance.

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