Dynamic laser speckle to detect motile bacterial response of
*Pseudomonas aeruginosa*

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**Abstract.** This proposal deals with the technique for detection of motile response of *Pseudomonas aeruginosa* using dynamic laser speckle or biospeckle as an alternative method. The study of bacterial displacement plays an essential role in biocatalysts processes and biodegradation. Hence, some biodegrading enzymes are benign catalytic that could be used for the production of industrially useful compounds as well as in wastewater treatments. This work presents an experimental set up and a computational process using frame sequences of dynamic laser speckle as a novel application. The objective was the detection of different levels of motility in bacteria. The encouraging results were achieved through a direct and non invasive observation method of the phenomenon.

1. **Introduction**
Bacterial motility is one of the most relevant subjects in pathogenesis and biodegradation areas. In this work we propose a methodology for the analysis of bacterial motile response using image sequences obtained with biospeckle laser technique.

Bacterial motility has long been suspected to be of importance in biodegradation [1] and pathogenesis of infections [2]. New knowledge on pathogenesis of bacterial enteric infections would be applied to new vaccine development, and comprehension of factors that enhance the transmission of pathogens [3].

The characterization of bacteria motile response has been approached with different optical techniques. Among them Schmidt *et al* [4] developed a laser-diffraction capillary assay to evaluate coefficients of bacterial random motility in semi-solid media. The Optical Coherent Tomography (OCT) was proposed by Wei Tan [5] as suitable to evaluate dynamic cell behavior. A diversity of cell processes, such as chemotaxis migration, proliferation, de-adhesion, and cell-material interactions, were characterized in thick tissue models. The images obtained by the OCT technique were compared...
with the images obtained by confocal microscopy (CM). Both techniques require complex and expensive equipment.

The granular pattern of high contrast that was discovered when a diffusion surface was illuminated by a laser beam was named "speckle" by early laser users. This irregular structure has been appropriately described through statistical and probability theory methods [7].

An interesting phenomenon was discovered when dynamic processes were observed with coherent illumination: the speckle patterns showed an active behavior. This phenomenon is originated by the light phase change interference produced by the movement of particles where the reflection takes place (scatterers). Speckle dynamic gives information of the speed of the center of sample scatterers [8]. It is a random pattern of interference that is described with statistical methods. Their properties and applications have been extensively treated in literature [9] - [12]. The speckles originated in biological samples are called biospeckles. Segmentation of image laser speckle regions based on its dynamics has been approached using specific algorithms [13] - [15].

Therefore, we can assume that the analysis of the speckle patterns could be considered a suitable tool to identify different degrees of bacterial motility. In this work, several results of dynamic laser speckle are shown to evince motile response of *P. aeruginosa* toward attractants.

An optical setup is proposed for acquisition, storage and processing of the speckle patterns image sequences. Several algorithms that have presented encouraging results in previous biological experiences, such as the detection of non visible bruising in apples and the viability of corn seeds [18] - [21], will be applied for processing the frame sets of bacterial biospeckles.

## 2. Materials and Methods

### 2.1. Culture of *Pseudomonas aeruginosa*

A strain of *P. aeruginosa* isolated from soil [22] was suspended into Mineral Salt (MS) liquid medium supplemented with triptone at a final concentration of 1% (w/v). After 24 hours of incubation at 25 °C with shaking at 120 r.p.m, two microlitres of this were inoculated into the centre of soft agar swarm plates containing MS medium plus LB (Luria-Bertani Broth) 1% (w/v), and 0.25% (w/v) agar. After 48 h of incubation at 25 °C in a wet chamber, bacteria were taken from the first originated swarm ring and resuspended into M S liquid medium plus Sodium Glutamate 1% (w/v). Then, mobile cells were grown aerobically at 25 °C overnight on a rotary shaker at 120 rpm.

After growth, cells were harvested by centrifugation at 3500 rpm about 15 minutes, washed once with sterile motility buffer, centrifuged again, and the precipitate was resuspended in motility buffer that had been previously vortexed to achieve good aeration. After 24 h with shaking at 120 rpm without any source of carbon and energy, aliquots (0.03 ml) were used for inoculating swarming plates for chemotaxis assays.

### 2.2. Biospeckle patterns acquisition

The proposed set-up for acquisition and storage of dynamic speckle patterns (biospeckle) is shown in fig 1. An expanded HeNe laser (632.8nm and 30mW) illuminates the plate under study from the bottom through a ground glass diffuser. A CCD camera connected to a frame grabber registers a sequence of images (8 bits and 768 x 576 squared pixels) and stores it into the computer. The CCD height and objective were adjusted to focus the sample.

The laser speckle technique was compared with the traditional technique to detect chemotaxis with white light, which consist of taking photographs of the plate illuminated from the bottom by a circular white light fluorescent tube. The photographs were taken by the CCD camera according the scheme of figure 1, where the diffuser was removed and the mirror was replaced by the fluorescent tube over a dark surface.

### 2.3. Processing biospeckle pattern sequences
Using the experimental set up of Fig. 1, sequences images of speckle patterns were recorded. Time series corresponding to intensity level of each pixel were assembled (as many series as the image resolution = 768 x 576 pixels) to study the dynamics of the phenomenon.

To evaluate the dynamic within stationary periods, images sequences of 400 samples were registered using a 4 Hz sampling frequency, during 1min 40s. To discover bacterial activity descriptors, three algorithms were assessed: the energy of the high frequency band, the entropy of the signal decomposition using the Discrete Wavelet Transform and the Generalized Differences.

2.3.1. High frequency band energy. The time intensity speckle patterns were previously normalized, dividing each one by its mean value, to minimize local differences due to reflectivity or sample illumination. Subsequently, they were filtered using a fifth-order high-pass Butterworth filter. The specifications of filter design were set as: maximum attenuation of 3dB within the band pass (above 1 Hz) and 30 dB as the minimum attenuation for frequencies lower than 0.5 Hz. The energy of the filtered signal was calculated with equation (1):

\[ E_{x,y} = \sum_{n=1}^{N} p_b(n)^2 \]  

(1)

Where \( p_b(n) \) is the intensity of the filtered signal corresponding to the \( x,y \) pixel location of image \( n \). Hence, a new image is built with the each pixel energy value, where the energy levels are correlated with bacterial mobility [22].

2.3.2. Wavelet entropy based descriptor. According to information theory, entropy is a relevant measure of order and disorder in a dynamical system. By using entropy, no specific distribution needs to be assumed. The spectral entropy as defined from the Fourier power spectrum shows a natural approach to quantify the degree of order of a complex signal, indicating the spread level of the signal power spectrum. The stationary condition to apply the Fourier transform (FT) is not ensured in the time speckle patterns. To deal with these limitations, time-evolving entropy can be defined from a time-frequency representation of the signal as provided by the discrete wavelet transform (WT). Previous works have reported encouraging results for the identification of biological dynamics with this tool [19], [23], [24]. In order to study the biospeckle, the time-series speckle patterns were divided

![Figure 1: Experimental set-up for acquisition of dynamic laser speckle patterns](image-url)
into \( N_T \) temporal windows of length \( L \). The energy of the detail \( j \) of WT decomposition, using Daubechies wavelet (order=2), was applied to obtain the window Shannon entropy (\( S_{WT} \)). The value was assigned to the window central point.

\[
S_{WT}^{(i)} = -\sum_{j=0}^{L} E_{j}^{(i)} \cdot \ln \left( \frac{E_{j}^{(i)}}{\sum_{j=0}^{L} E_{j}^{(i)}} \right) 
\]  

The mean energy of the WT \( j \) coefficients in each window \( i \) is obtained using equation (3):

\[
E_{j}^{(i)} = \frac{1}{N_j} \sum_{k=0}^{(L/2)-1} |C_{k,j,l}|^2 
\]

with \( i =1, \ldots ,NT \).

The \( S_{WT} \) entropy has been proposed in previous works to characterize the biospeckle phenomenon in images sequences that show inhomogeneous activity within different regions [19]. A new image is generated with \( S_{WT} \) values. In these experiments, a set of 150 images were registered every two hours during 24 hours. The \( S_{WT} \) images give time and space information of bacterial chemotactic response.

2.3.3 Generalized differences. A qualitative technique used to analyze speckle patterns are the Generalized Difference (DG) method [12]. It assigns the image pixel value as the sum of intensity differences between each possible pair of the ensemble.

\[
GD(x, y) = \sum k \sum l |I_k(x, y) - I_{l,j}(x, y)| 
\]

Where \( I_{(x,y)} \) is the intensity of \( x \) and \( y \) coordinates, \( k \) and \( l \) are the images indexes and the bars indicate the absolute value. In previous works the intensity levels of the resulting image have shown positive correlation with the dynamics associated to the space region.

3. Results

The achieved results are outstanding elements for evaluation and processes of laser biospeckle patterns experiments. Wells swarming assays were used for bacterial motility assessment, they were inoculated with cultivated \( P. \) aeruginosa during 24 hs, incubated 25\(^{\circ}\) C, and the rings detection was evaluated in intervals of 24 h. In figure 2 and figure 3 a four well assay is shown, left ones (upper and lower) with positive attractant (Tryptone 1% W/V) and those on right side with buffer solution as negative control without carbon source. Figure 3 shows the process of images sequences using wavelet entropy based descriptor, 48 h and 72 h after inoculation.

4. Discussion

By analyzing figure 2, it can be inferred that the high frequency band energy algorithm performs a better discrimination between different bacterial activity regions than the generalized differences algorithm.

In figure 3, results of the wavelet entropy based descriptor process are shown. In addition to the fact that its performance is quite similar to that obtained with the high frequency band energy; this algorithm is suitable for analyzing the underlying dynamics of a changing phenomenon, because it performs the process of continuous time windows. In consequence, it is possible to generate videos by assembling "activity" images to observe bacterial motile response appropriately.
5. Conclusions
In this work the acquisition and process of laser biospeckle patterns to assess bacterial motile response has been proposed. The encouraging results showed the efficacy of this method, whereas the pattern analysis allowed distinguishing the bacterial mobility regions.

The detection of bacterial displacement at macroscopic level displays well-known difficulties. The traditional observation method with white light does not allow for discerning motile from non motile bacteria clusters. Besides, it also presents disadvantages such as low sensitivity at reduced population density and inability to segment regions with different bacterial activity levels (motility). In connection with the proposed algorithm to process biospeckle patterns, the so-called Generalized Differences have shown lower sensitivity than the energy of the high-frequency band to detect bacterial motility.

To monitor continuously a bacterial motile response, the Shannon entropy based in wavelet decomposition is recommended. This approach is adequate for evaluating non-stationary processes. In this particular application a video, compiled with the entropies images calculated with sliding windows, could give time-space information of bacterial displacements.

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