Use of a laser speckle system in the determination of antibacterial susceptibility by the disc diffusion method

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Introduction

Purpose of the study.
Rapid identification of antibacterial resistance plays a crucial role in treatment of acute infectious conditions, Early identification of infectious agent and its sensitivity to antibiotics can provide targeted pharmacological intervention at the early stage of the disease, increasing patient's survival. The issue has become relevant in the treatment of elderly patients. The empiric antimicrobial treatment must be replaced as soon as possible to reduce the risk of mortality of patients. Phenotypic resistance tests such as disk diffusion and E-test require 16-24 hours to obtain the results [1], while the PCR tests provide only genotypic type of antibacterial resistance.

Suggested method.
In previous studies using laser speckle imaging technique, we managed to determine the bacteria growth after 2-3 hours from the beginning of activity [2]. In the laser speckle imaging method, a laser beam is scattered on a Petri dish where the test bacteria and antibiotic discs are located. The laser speckles reflected from the surface are recorded sequentially in time. A sub-pixel correlation analysis was proposed to detect small changes in the sequence of laser speckle images, and the effects associated with changes in bacterial activity can be observed.
Experiments were performed at the Pauls Stradins Clinical University Hospital Joint Laboratory on different bacteria and their corresponding antibiotics [3].

The experimental setup.
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 (Fig.1).

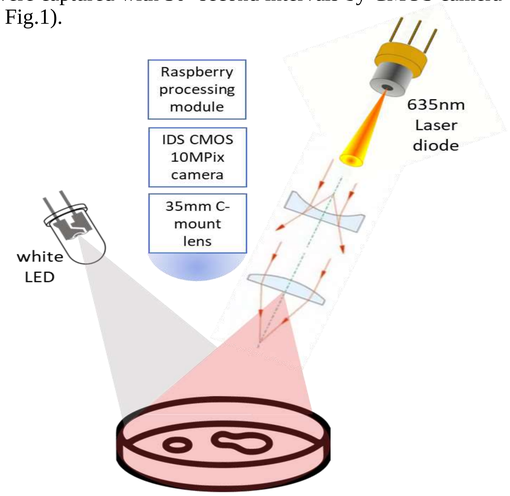

Figure 1. Setup scheme for burst image capturing of bacteria growing process under 635 nm laser and white LED illumination.

Algorithm

Algorithm description
1) The entire field of the experiment was divided into NxN pixels sections. The following steps are performed for each section separately.
2) Two-dimensional normalized correlation between consecutive images was performed.
3) The value of offset was found at the location of the correlation peak. This value characterizes the changes that occur between frames.
4) To find a more accurate value of the offset, interpolation was performed within the maximum of the correlation function.
5) Offsets obtained between each pair of adjacent samples were accumulated.
6) To avoid the influence of local transient spikes, a signal envelope within a certain window was used.

Performing the described algorithm between each pair of consecutive frames for the entire sequence creates a "time signal". An increase of signal values will be observed when bacterial growth occurs.

Algorithm Benefits.
1) By sensitive correlation subpixel analysis, the changes in bacterial activity can be detected
2) The subpixel correlation technique allow to detect submicron bacterial events earlier (Fig.2).

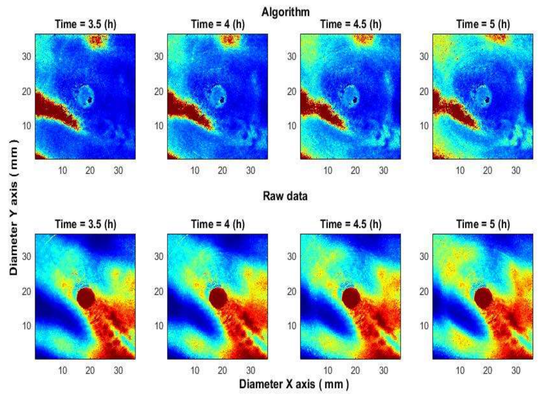

Figure 2. The images in the top row show the result of the correlation analysis, while the images in the bottom row show the raw data.

Results

As a result, it was obtained that after a few hours from the start of the experiment a disc of the sterile zone begins to form around the antibiotic. The sterile zone becomes clearly visible within a couple of hours after the beginning of formation. The result was obtained significantly earlier than by the disk diffusion method [4] for the same bacterial species [4]. Creating a spatio-temporal image averaging around each radius as it moves away from the center (from the antibiotic), we can obtain a certain curve, similar to the letter "S", according to which the size of the sterile zone changes over time (Fig. 3). This behavior has been observed in several different experiments.

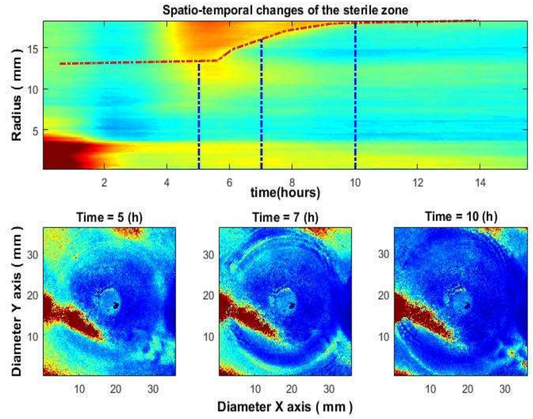

Figure 3. Spatio-temporal changes of the sterile zone (top), and sterile zone formation in the growth of *Acinetobacter baumanii* around the imipenem disc (bottom)

Conclusions

The obtained results make it possible to predict the diameter of the sterile zone around the antibiotic disk faster than using the disk diffusion method without laser speckle technology. The practical implementation of the obtained technology will allow the laboratory to send the results and their interpretation to the clinic much faster.

Bibliography

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