New Results and Trends in Interferometric Observation of Bacterial Activity

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Abstract

The metabolic activity of Bacilo Calmette-Guérin (BCG) was observed monitoring small changes of the refractive index of the nutrient solution interferometrically. Two samples were kept in adjacent compartments of a stainless steel sample holder, which was equipped with optical windows. One sample contained 2.5 ml of the nutrient solution (Bacto Middlebrook 7H9 Broth with ADC Enrichment) and $4x10^6$ bacteria BCG. The other one, which was used as a reference, contained only the nutrient solution. The sample holder was put between the mirrors of a Fabry-Perot type interferometer. The light source used was a polarized He-Ne laser and the primary laser beam was split into two parallel beams to provide simultaneous measurements of both samples. The temperature of the sample holder and interferometer was computer controlled with precision of e few mK. A temperature scan technique was used to resolve interference fringes with a resolution better than 3% of a fringe. This, in terms of index change, corresponds to a resolution of $2x10^{-7}$. The interferometer is stable during weeks. In the present work results of new measurements are presented and a new kind of interferometer, which is being built, is described, were the temperature controlled phase scan is substituted by a spatial phase scan. This technique permits even higher phase resolution and reduces the time of data analysis considerably. Observation of bacterial activity is used to determine susceptibility of bacteria against antibiotics. The conventional way uses cultures on solid substrates and is extremely time consuming, which can be prohibitive in certain cases. There exists a method to observe bacterial activity in real time that uses ^{14}C labeled nutrients (BACTEC 460TB). However, this method is so expensive that its use is restricted to very few research laboratories. The interferometric technique is a low cost alternative to the radioactive method.

Introduction

Despite the invent of antibiotics, bacterial diseases still constitute a major problem for mankind. The treatment of diseases, such like tuberculosis, usually stars with tests of bacterial susceptibility against antibiotics in order to permit a specific treatment of the patient. Experiments looking for antibiotic activity against bacteria are usually performed *in vitro* with disc antibiogram method or *in vivo* through accounting of Colony Forming Units (CFU) of the chosen organ [1]. These investigation methods consist of the observation of bacterial colonies formed from single cells. Both methods are time consuming and do not allow to observe the immediate proliferation of bacteria in real time. For example, observation of first colonies in culture of *Mycobacterium tuberculosis* through CFU method takes typically 5 to 16 days [1]. Another method, which permits to observe bacterial activity in real time, is the well known radiometric method (BACTEC 460 TB), which uses ^{14}C marking. However, the use of radioactive nutrients limits this method to a few research laboratories [2]. In the present work a cheap method to monitor the metabolic activity of micro-organisms in real time is described. The method uses precision measurements of small changes of refractive index of the nutrient solution.

Experimental Setup

The measuring principle consists of an interferometric measurement of small changes of the refractive index of the nutrient solution due to the metabolism of the micro organisms. The sample is kept in a stainless steel vessel of 46.55 mm length that can be sealed hermetically and that has two entrance glass windows. This sealed sample is put into an interferometer. The experimental results described here were obtained with an interferometer with two parallel glass-air interface reflectors. As described at the end, for future experiments a folded Michelson interferometer will be used. The primary light source was a polarized He-Ne laser. In our experiments the changes of refractive index due to bacterial metabolism are typically of the order of 10^{-5}. This value is so small that temperature changes as small as 0.01K would seriously interfere in the measurement. In principle it would not be too difficult to control temperature with such precision. However, it turned out to be of grate help to

permit small temperature variations, which create additional interference oscillations, and to subtract the corresponding index change. This way a small index change caused by metabolism, which would result only in a single oscillation, may be monitored with many intermediate data points taken with high precision from the maxima and minima of the additional oscillations caused by temperature change. In order to be able to perform a reliable subtraction of the temperature effect and other possible index changes, such as chemically induced ones, the sample holder is equipped with a second compartment that contains the pure nutrient solution without bacteria. A second identical interferometric measurement is performed simultaneously with this reference liquid, giving rise to a reference signal. In order to be able to subtract the reference phase change with the correct sing temperature is being monitored during the whole experiment. The sample holder is kept in a water bath inside a thermal insulation. The stainless steel sample holder acts as an isothermal block. Two incandescent lamps are used for heating the bath. Temperature was controlled with the same computer that was used for data-acquisition.

Data analysis and results

The experiments performed in this work used Bacilo Calmette-Guérin (BCG) (strain Pasteur), which is a non-virulent mycobacterium obtained from sequenced cultures of Mycobacterium bovis [3]. The BCG is a strictly aerobic species, which floats on the sample surface [4]. The probing laser beam passes a few millimeter beneath the bacteria containing surface so that the changes of concentrations due to metabolism are brought to the measuring beam by diffusion with relatively small delay. The culture media used were Bacto Middlebrook 7H9 Broth with OADC Enrichment.

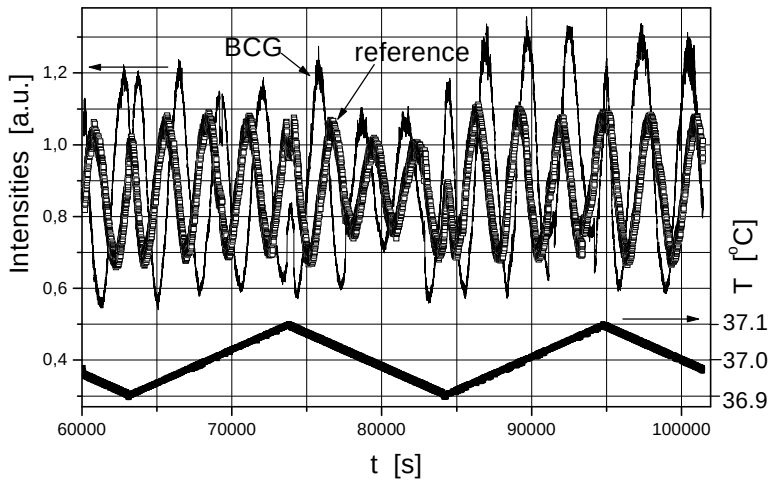

Fig. 1 Typical signal of light intensities and temperature.

Fig. 1 shows a typical signal of the light intensities registered from the signal and reference beam, as well as the recorded temperature. The temporal position of the maxima and minima of light intensity are determined by means of quadratic fittings around the extrema. From these times and the information on the sign of temperature change two curves of light- phase versus time are constructed. Changes of the light phase are finally converted into changes of refractive index. Figure 2 shows the difference of refractive index of the sample and the reference solution as a function of time.

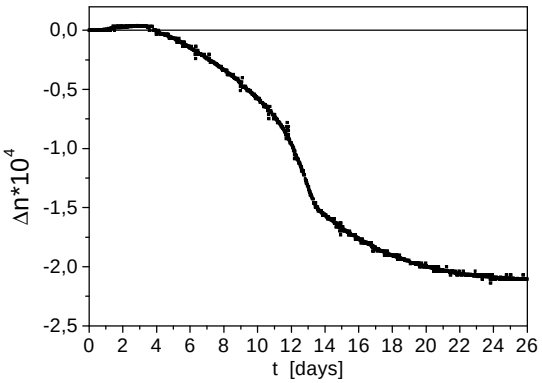

Fig. 2 Refractive index change caused by bacterial metabolism.

The metabolism of BCG is expected to decrease the refractive index of the nutrient solution In deed, as can be seen from figure 2, after 3 days, when the log phase is expected to start, the refractive index decreases, roughly following an exponential growth law with a time constant of about two days. After 13 days the exponential growth turns over into an exponential decay law (time constant 4.6 days and final index change $\Delta n = -2\text{x}10^{-4}$) . This saturation is probably due to depletion of oxygen in the sample compartment.

Experiments with bacteriostatic substances have also been performed and the biological response is clearly detected with the interferometric method.

Future Modifications of the Interferometer

The present equipment has several drawbacks: a) only a single sample can be measured at a time, b) the data analysis is extremely time consuming, c) the small temperature variation during temperature controlled phase scan creates tiny temperature gradients in the interfeometer, which limit the sensitivity of the instrument. Therefore it is planned to abandon the temperature controlled phase scan. In the new scheme the interfering beams are not 100% parallel so that a spatial fringe pattern is formed. This pattern can be read by a CCD camera and the phase shift due to index changes can be determined from these images automatically. The interferometer will be constructed such that temperature changes cancel out to first order. Instead of using an interferometer with a fix reference phase the reference solution will be used to generate the reference phase. This way no subtraction of the signal form the reference measurement is necessary. It is planned to use a Michelson interferometer for this purpose, were one of the arms will be folded such that the sample and reference solution may be kept close to each other. Experiments are performed to find a cheap way of examining several samples simultaneously.

Conclusions

It has been shown that interferometric measurements of small refractive index changes can be used to monitor the activity of slowly growing bacteria like BCG. This method has potential biomedical applications and may substitute the expensive radiometric methods of bacterial susceptibility measurements.

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