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ESTIMATION OF DETECTION LIMITS OF THE PHAGE-INVASION BASED IDENTIFICATION OF BACTERIA

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Recently a new method, SEnsing of Phage-Triggered Ion Cascade (SEPTIC) was proposed for the rapid detection and identification of bacteria via the electrical field caused by the stochastic emission of ions during phage infection. In this Letter, we present linear network theoretical considerations about the detection limits of the method. The considerations are based on our published data of the *E. coli* detection experiments and on the assumption of a linear response between the number of bacteria and the measured power density spectrum of the fluctuation-signal. Some practical limits of the detectability of the present agents with possible noise measurement arrangements are discussed in this paper. The calculations indicate that the detection and identification of a *single* bacterium can be achieved with natural (wild) phages with reasonable efforts within a time window of 10 minutes.

Keywords: Prompt identification of bacteria; detection limits; nano-bio chip; phages; noise.

1. Introduction

Very recently, a method of prompt detection and identification of bacteria was proposed [1]. The new method, SEnsing of Phage-Triggered Ion Cascade (SEPTIC), is detecting and analyzing the electrical field caused by the stochastic emission of ions during phage infection. The detection and identification is done by measuring the microscopic voltage fluctuations in a nano-well device [1,2], and it takes less that 10 minutes. Because the

phage infection is a very selective process, where only bacteria of a specific strain are infected, a SEPTIC-biochip [2] containing an array of sensors where each sensor is sensitized with a different phage can detect and identify all relevant bacteria with the extraordinary speed and selectivity. In this paper, we present some considerations about the sensitivity of the method.

2. Noise Circuitry of the Measurement Setup

The noise circuit diagram of the SEPTIC measurement setup is shown in Fig. 1. This model circuitry is simplified in accordance with the low-frequency range of the investigations (<10 Hz). The resistance R_{nw} of the nano-well is about 1 MOhm. S_{nw} and $S_{u,n}$ are the power density spectra of the nano-well signal voltage and the input noise voltage generator of the preamplifier, respectively. $S_{i,n}$ is the power density spectrum of the input current noise of the preamplifier. During the experiments and at any foreseeable/reasonable low-frequency arrangements in the future, the input impedance of the amplifier can be neglected. Thus, the equivalent input voltage noise spectrum [2] can be given as:

$$S_{u,eq}(f) = S_{nw}(f) + 4kTR_{nw} + S_{u,n}(f) + R_{nw}^2 S_{i,n}(f), \tag{1}$$

where the S_{nw} has a $1/f^2$ shape. In the relevant frequency range (<10 Hz), $S_{u,n}$ and $S_{i,n}$ expected to have $1/f$ shape and R_{nw} is independent of the frequency.

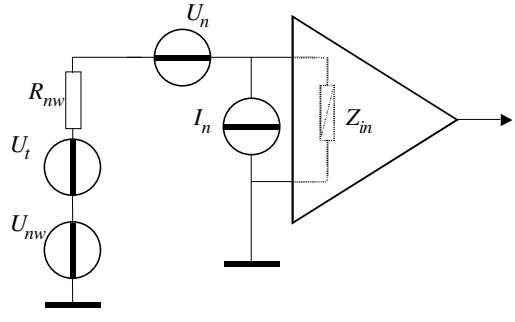

Fig. 1. Simplified noise circuitry of the nano-well arrangement. U_{nw}, U_t and R_{nw} are the signal voltage generator, the thermal noise voltage generator and the internal resistance (1 MOhm in the experiments) of the nano-well, respectively. U_n and I_n are the input noise voltage and input noise current generators of the preamplifier, respectively. Z_{in} is the input impedance of the preamplifier (100 MOhm in the experiments, thus it can be disregarded).

3. Estimation of Detection Limits

The results are presented in Fig. 2. During the experiments [1], 10^7 bacteria were present in the total sample volume of 10mm^3. Based on Eq. 1 and the experimental data [1], the detection limits of the number of bacteria are estimated at frequency 1 Hz. The data published in [1] are obtained at the total bacterium count of 10^7 and at 5 minutes incubation time.

The rest of the curves are calculated by assuming a linear response in the power density spectrum of the signal against the number of bacteria. This assumption is justified

by the facts that the signal is stochastic and the bacteria act as independent sources of the total fluctuations. It is however an important precondition that, while the bacterium concentration may be low, the probability of infection of a given bacterium should be the same as at high bacterium concentrations. This important condition is naturally satisfied by using a fixed number N_p of phages, where N_p is much greater than the highest possible number $N_{b,max}$ of bacteria. At the experiments, the N_p was $10*N_{b,max}$. The numbers of bacteria are valid for 10 mm^3 fluid, which is the practical experimental condition set by the smallest drop volume (5 mm^3) and the need of mixing two fluids (bacterium and phage containing solutions).

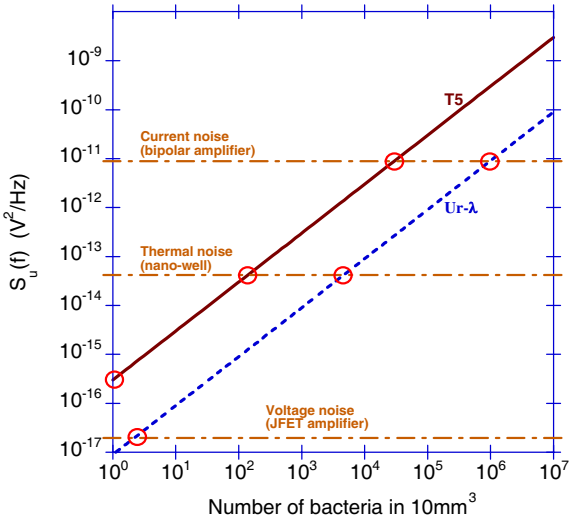

Fig. 2. Estimation of the ultimate limits of detectability of *E. coli* with the tested phages and various measurement arrangements. A linear response is assumed. The various detection limits are indicated by circles where the X coordinate of the center of the circle is the limit.

The highest limit is set by the actual conditions of the published experiments. This limit is provided by the input current noise of the bipolar preamplifier which makes the last term of Eq. 1 dominant among terms 2-4. We used the highest 1/f noise level measured at the negative experiments to determine this limit. The corresponding input current noise is about 10^{-23} A^2/Hz which is consistent with the amplifier data at 1 Hz. This mechanism sets the detection limits to about 30,000 bacteria with the T5 and 1 million bacteria with the Ur – λ phages.

A significantly lower limit is set by the thermal noise. This limit can easily be reached by a JFET preamplifier which has negligible current noise (about 10^{-30} A^2/Hz). That makes the last term of Eq. 1 the smallest for JFET low-noise preamplifiers. In this case, the dominant term among terms 2-4 in Eq. 1 is the 3rd one. The limit is obtained supposing 1 MOhm for R_{nw}. This mechanism sets the detection limits to about 200 bacteria with the T5 and 5000 bacteria with the UR – λ phages.

The most enhanced sensitivity could be achieved using thermal noise reduction techniques, such as cross-correlation type nano-well and preamplifier arrangements and reducing the conductivity of the fluids thus reducing R_{nw}. The last action may decrease the sensor response too; however, the probability of this is vague because the Debye

screening length of deionized water is only 1 micron and the mean distance between bacteria in the experiments is 10 micron, which indicates that the observed effect is not a long-range one. Thus decreasing the Debye length by increasing salt concentrations will most probably have a negligible effect on the induced electrical field fluctuations. In the most optimal case, if the thermal noise can be eliminated by conductance increase so much that the second term of Eq. 1 becomes the dominant, we can reach the highest sensitivity shown in Fig. 2. Typical voltage noise data of low-noise JFET amplifiers are used. This mechanism decrease the detection limits to 1 bacterium with the T5 and 2 bacteria with the $\mathrm{Ur}-\lambda$ phages. By using cross-correlation technique this limit can further decreased.

Note, if the conductivity of the fluid cannot be increased, it is still reasonable to suppose that we can reduce the thermal noise by cross-correlation technique and optimized nano-well geometry by a factor of 100. That achievement would provide detection limits of 1 bacterium with the T5 and about 30 bacteria with the $\mathrm{Ur}-\lambda$ phages.

4. Conclusions

It was shown by linear circuit theory and supposing linear response that, by using low-noise JFET preamplifiers and 10 mm^3 sample droplet, the detection limits of *E. coli* are about 200 bacteria with the T5 and 5,000 bacteria with the $\mathrm{Ur}-\lambda$ phages, respectively. It was also shown that we could reasonably suppose that the application of increased fluid conductivity and/or more sophisticated techniques, such as cross-correlation arrangements, can decrease the detection limit to 1 bacterium.

The significant difference between the detection limits of E. coli using T5 versus Ur-lambda phages, respectively, may be due to the fact that the adsorption of Ur-lambda is temperature sensitive. However, all the measurements, except for the preincubation time, were carried out at room temperature, below the optimal 37°C. Thus, in a practical sensing arrangement, keeping the chip temperature at 37°C may imply a significant enhancement of sensitivity.

Note, there are also other tools, which may enhance sensitivity, such as methods of driving the bacteria to the nano-well surface, or nano/micro-fluidics to accelerate the dynamics of the system. These techniques may be needed if, at low concentrations, the dynamics of the response slows down and too long measuring times are necessary to get stationary data at low concentrations. This possibility is an open question at the moment and further experimental and theoretical efforts are necessary to clarify the situation.

Acknowledgments

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References

[1] M. D. King, S. Seo, J. U. Kim, R. F. Young, M. Cheng and L. B. Kish, Rapid Detection and Identification of Bacteria: SEnsing of Phage-Triggered Ion Cascade (SEPTIC) *J. Biological Physics and Chemistry* **5** (1), in press.

[2] C. D. Motchenbacher & J. A. Connelly, Low-noise electronic system design, *John Wiley & Sons*, New York (1993).