Towards biological quantity theory for nominal property metrology in polyenzymatic devices with living cells

P I Belobrov[1], A A Evstrapov[2], E N Esimbekova[1,3], I A Denisov[1,4], K A Lukyanenko[1,4], E D Osipova[5], A S Yakimov[5]

1 Laboratory of Bioluminescent Biotechnology, Siberian Federal University, Krasnoyarsk, 660041, Russia
2 Institute for Analytical Instrumentation RAS, St. Petersburg, 190103, Russia
3 Laboratory of photobiology, Institute of Biophysics SB RAS, Krasnoyarsk, 660036, Russia
4 Laboratory for Digital Controlled Drugs and Theranostics, Federal Research Center "Krasnoyarsk Science Center SB RAS", Krasnoyarsk, 660036, Russia
5 Research Institute of Molecular Medicine and Pathobiochemistry, Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, Krasnoyarsk 660022, Russia

peter.belobrov@gmail.com

Abstract. The current metrological revolution (16/11/2018 - 20/5/2019), along with the quantum system of units of physical nature, initiated the transfer of the problem of understanding non-physical variables beyond quantitative biology. A number of modern biological concepts require an adequate mathematical description in the language of biological spaces, topologies, and measures. Examples include such objects as microbiomes, cells, aptamers, etc., along with processes of biological action: gastrulation, development, heredity, etc. Here we discuss the concepts of "biological quantity" and "nominal property" within the framework of the problem of biological measurements based on new specific results of biological analysis using a microfluidic platform and chips developed by our team earlier. It was shown that based on different microfluidic platforms it is possible to develop chips with a polyenzymatic bioluminescent system NAD(P)H:FMN-oxidoreductase-luciferase (Red + Luc), which can be used in various areas of biological analysis, for example, environmental bioassay. Thus, disposable microfluidic chips with Red + Luc system suitable for field and indoor use were developed using continuous flow microfluidic platform. The use of droplet-based microfluidic platform allowed to develop microfluidic chips with Red + Luc system for long-term continuous measurements of water samples, for example, in places of waste disposal by industrial enterprises or water treatment plants. The reference for comparing different biological quantities with each other in the proposed system was a photodetector, which converted non-numeric values and nominal properties recorded by a biological module Red + Luc into numerical variables. Such a reference was implemented as a portable luminometer based on silicon photomultiplier. The results allow to perform other biological measurements and to start the discussion of modern biological concepts in the language of biological measures that are useful for novel version of metrological dictionary (VIM4).

1. Introduction

Galilei created fundamental physics by formalization of physical phenomena four centuries ago. The great final is the system of quantum units of Nature was adopted at 16/11/2018 at 26th meeting of the CGPM without any artefacts. A matter-of-course the question arises: "what Nature?" with exact answer: "of course, physical!" The chemical and, especially, biological description of Nature contain much more non-quantitative values having the qualitative character. The problem is reflected in metrological concept "nominal property", which is written "propriété qualitative" in French and in Russian has many meanings: "formal, nominal, and specified or indicator property". Therefore, the decade of joint discussion the “biological quantity” and "nominal property" allow formulating the problem of biological measurement [1, 2, 3].

The traditional approach to measuring biological variables is to apply physical measures to biochemical processes. More complex than “depletion interaction” was introduced “biophysicochemical interaction” [4]. Bio-numbers [5] and quantitative biochemistry in living cells [6] were born. Metrological results of this direction reflected in the new edition of the metrological dictionaries [7, 8].

We think that any cell measures the other cells (without microscopes and micrometers!) in the cell-to-cell communication by membrane-derived vesicles and exosomes with different kinds of molecules (chemical signals). Theoretical description of those processes is “cell-to-cell measurement”. The qualitative variables can be quantified in the space of infinite dimension by recurrently perhaps. Therefore, we must here note that the movement to this limit will always be through more and more ingenious formalizations using new physical, chemical and biological measures [9, 10].

As all cells, tissues, organs etc. are differ from each other, as we cannot directly use any cell etc. as reference. Luca Mari suggested good idea for novel treatment of nominal properties in biological metrology when we apply some measurement procedure for a comparison [11], i.e. quantify of biological objects and processes. Our approach is to develop biological measures and digitize those using microfluidic technologies and devices.

Microfluidic platform is basis of microphysiological system development [12, 13, 14]. "Organs on chips" and "tissue-engineered organ constructs" have direct application to the rapidly developing field of systems biology attempts to span scales with integrative, bottoms-up modeling coupled with massively parallel biological measurements [12].

Here we briefly describe our results in application of polyenzymatic chains with bacterial luciferase with different microfluidic technologies for biological measurements in the scope of biological metrology [15, 16, 17, 18, 19].

2. The choice of the converter of biological signals into physical

In the description of the physical phenomena of nature it is common to use the fundamental physical constants as reference parameters for comparison and interpretation of experimental data. These constants, for example, include the Avogadro constant N_A, the Boltzmann constant k, the Planck constant h, and others. The interaction of biological objects (organisms, tissues, cells) among themselves is much more complicated than the interaction of objects of a purely physical nature. When describing this interaction, it is also necessary to take into account new properties and biological variables (including non-numerical) that occur in interacting objects under the influence of self-organization and the desire for biodiversity. For example, there are no two absolutely identical cells in nature. In this connection there is the problem of choosing the biological converter of non-numeric variables into numerical variables which can be measured by physical methods.

Enzymes can be one of the possible biological converters. Unlike cells, enzymes after synthesis always have the same structure and physicochemical properties. Errors in the structure of the enzyme during its synthesis do not allow it to adopt the correct conformation, which excludes it from strictly aligned biological chains of interaction of organisms. At the same time, enzymes, being biological objects, can be used as indicator systems when describing the interaction of biological objects with each other or with the environment.

Luciferase enzyme is an example of such a biological converter. This enzyme is bioluminescent, that is, during a chemical reaction with its participation, light quanta (hν) are emitted, as shown in the reaction scheme below. Here we used polyenzymatic bioluminescent system NAD(P)H:FMN-oxidoreductase-luciferase (Red + Luc):

$$FMNH_2 + RCOH + O_2 \xrightarrow{\text{Luciferase}} RCOOH + H_2O + h\nu \quad (1)$$

$$NADH + FMN + H^+ \xrightarrow{\text{NAD(P)H:FMN-oxidoreductase}} FMNH_2 + NAD^+ \quad (2)$$

Luciferase catalyzes the oxidation of aldehydes with the emission of light in the blue-green spectral region (1). The luciferase reaction is coupled with reaction (2) catalyzed by NAD(P)H:FMN-oxidoreductase in order to provide luciferase with reduced flavin mononucleotide ($FMNH_2$).

Light quanta emitted during a chemical reaction can be accurately counted using photo detectors. In this work, we used silicon photomultiplier (SiPM), which allow us to register individual photons of light. Moreover, SiPM possesses a compact size that allows the development of portable measuring systems on its basis.

3. Biological measurements in microfluidic chips with Red + Luc system

On the basis of Red + Luc bioluminescent system, photodetectors and various microfluidic platforms, portable biosensors were developed for various biological measurements. Here we briefly describe these biosensors and discuss their application as a reference measuring systems for biological parameters.

3.1. Disposable polymer microfluidic chips

Previously we demonstrated [17] the integration of Red + Luc system with microfluidic platform of continuous flow. For that the surface of PMMA (Poly(methyl methacrylate)) substrate was channelized followed by immobilization of Red + Luc system in the reaction chamber and then sealed using solvent bonding. The water sample added through the inlet channel dissolved the components of Red + Luc system immobilized in reaction chamber of the chip and, thus, bioluminescent reaction started. These chips were tested for application in environmental bioassay. The proposed indication system demonstrated limit of detection for copper (II) sulfate at the level of 3 μM which was comparable with copper (II) sulfate LOD of traditional lux- biosensors based on bacterial cells (1 μM). Moreover, analysis time with disposable microfluidic chips was reduced from 3 to 5 h with cell-based bioassays to 1–3 min.

Then the integration of luciferase-based microfluidic chips with a portable Luminometer was performed in order to develop handheld enzymatic luminescent biosensor (Fig. 1) [19]. For that the composition of Red + Luc system was optimized to achieve higher luminescence intensity and sensitivity for toxic compounds. The detection system of the portable luminometer was based on a SiPM PM6650-EB from KETEK.

This biosensor was suitable for field and indoor use. For example it could be used as an early warning field-deployable system for rapid detection of heavy metals salts and other toxic chemicals, which affect bioluminescent signal of enzymatic reaction.

3.2. Microfluidic chips for continuous biological measurements

Another biosensor was made using droplet-based microfluidic platform. For that a polydimethylsiloxane (PDMS) chip was designed and manufactured using soft lithography techniques. Photomultiplier tube H7828 from Hamamatsu was used as a photodetector. The Red + Luc system was split into two solutions which mixed and formed oil-in-water emulsion with droplet microreactors (see description of Fig. 2).

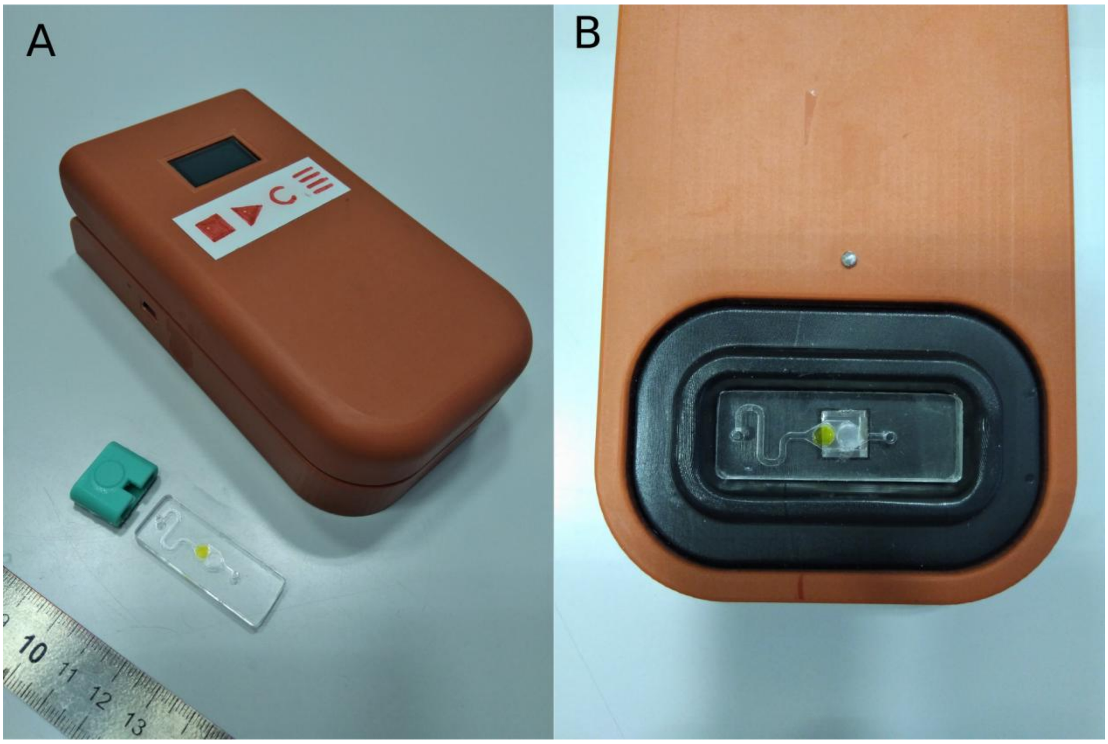

Figure 1. The handheld biosensor consisted of a portable SiPM-based luminometer, disposable microfluidic chips with immobilized bioluminescent enzyme system and a sampler adapter for chips [19].

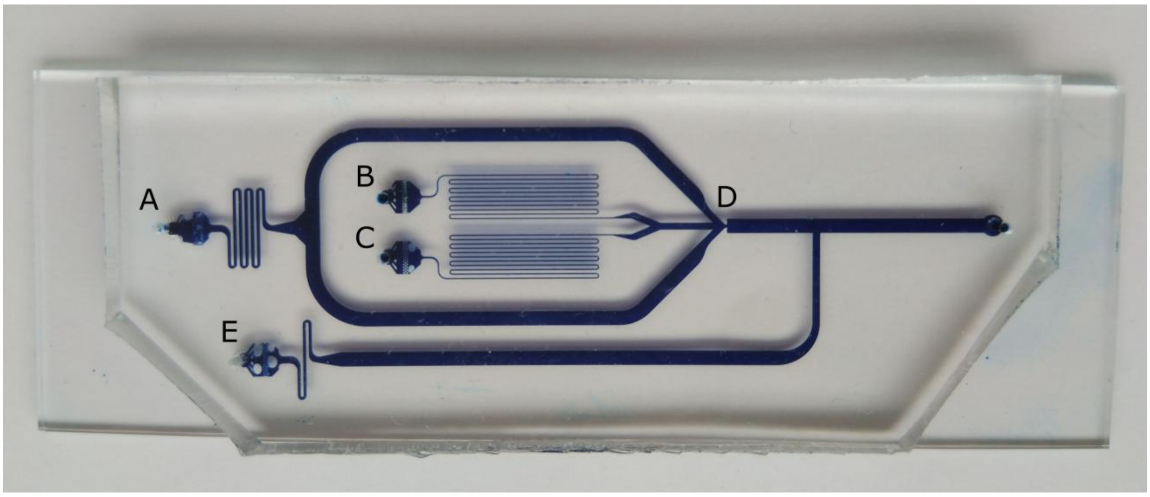

Figure 2. Overview of PDMS chip for Red + Luc system based on droplet microfluidic platform. Red + Luc system was fed into the chip in the form of two solutions. One solution (B) contained enzymes and NADH. The other solution (C) contained aldehyde, FMN, and the analyzed sample. After merging of B and C, the flow is compressed by the oil phase (A) at the point of hydrodynamic focusing (D) forming water-in-oil emulsion. Water droplets contained equal volume fractions of B and C. The volume of oil phase (E) was additionally introduced into the flow between the droplets to increase the distance between them and mix reagents. After passing the additional channel (E), the content of the droplets was mixed and the bioluminescent reaction started. Droplets passed through the capillary of the chip opposite the aperture of the photodetector, where the signal was recorded.

Mathematical modeling of the formation of droplets with a volume of 0.1–10 μl showed that the distance between adjacent droplets is much smaller than their diameter; therefore, an additional oil channel was introduced into the design of the microfluidic chip, allowing to adjust the distance between the droplets. Also, computer simulation showed that the deformation of droplets near this channel led to effective mixing of the reactants, which was confirmed experimentally.

The developed chip was tested for application in environmental bioassay as the disposable one. Results indicate that long-term continuous measurements in droplet microreactors with Red + Luc system reached 1 mg/l LOD for copper (II) sulfate, which was at the MPC level. Also at the level of the MPC (0.2 mg / l), it was possible to detect benzoquinone in water.

The application of droplet-based microfluidic platform allowed to develop microfluidic chips with Red + Luc system for long-term continuous measurements of water samples, for example, in places of waste disposal by industrial enterprises or water treatment plants.

4. Conclusions

In this paper, a mechanism for conversion of non-numerical biological variables into measurable numerical variables on the example of environmental bioassay was proposed. Enzymes, in particular luciferase, were proposed to use as a biological converter. This approach allows to take a fresh look at the problem of searching for a biological reference to perform biological measurements and to discuss modern biological concepts in the language of biological spaces, topologies, and measures that are useful for novel version of metrological dictionary (VIM4).

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