Bioluminescence for Food and Environmental Microbiological Safety
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Introduction to the Series

Since its conception in 1989, the Tutorial Texts series has grown to more than 70 titles covering many diverse fields of science and engineering. When the series was started, the goal of the series was to provide a way to make the material presented in SPIE short courses available to those who could not attend, and to provide a reference text for those who could. Many of the texts in this series are generated from notes that were presented during these short courses. But as stand-alone documents, short course notes do not generally serve the student or reader well. Short course notes typically are developed on the assumption that supporting material will be presented verbally to complement the notes, which are generally written in summary form to highlight key technical topics and therefore are not intended as stand-alone documents. Additionally, the figures, tables, and other graphically formatted information accompanying the notes require the further explanation given during the instructor’s lecture. Thus, by adding the appropriate detail presented during the lecture, the course material can be read and used independently in a tutorial fashion.

What separates the books in this series from other technical monographs and textbooks is the way in which the material is presented. To keep in line with the tutorial nature of the series, many of the topics presented in these texts are followed by detailed examples that further explain the concepts presented. Many pictures and illustrations are included with each text and, where appropriate, tabular reference data are also included.

The topics within the series have grown from the initial areas of geometrical optics, optical detectors, and image processing to include the emerging fields of nanotechnology, biomedical optics, and micromachining. When a proposal for a text is received, each proposal is evaluated to determine the relevance of the proposed topic. This initial reviewing process has been very helpful to authors in identifying, early in the writing process, the need for additional material or other changes in approach that would serve to strengthen the text. Once a manuscript is completed, it is peer reviewed to ensure that chapters communicate accurately the essential ingredients of the processes and technologies under discussion.

It is my goal to maintain the style and quality of books in the series, and to further expand the topic areas to include new emerging fields as they become of interest to our reading audience.

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Preface

The threat of biological warfare and the demand for safer food and water are an increasing concern. The best defense against bacterial infectious agents is their early detection and/or identification. Time and detection sensitivity are crucial for this type of microbiological analysis.

Besides the social aspects, successful competitiveness in international food markets increasingly depends on the provision of fresh, wholesome, and safe food. The accepted strategy worldwide is to prevent food and environmental contamination at the early stages of the food chain rather than to test the final product and, in case of failure, recall it. The control program used by most food producers worldwide and required by food inspection agencies in most of developed countries is the Hazard Analysis and Critical Control Point (HACCP) system. HACCP is a management system with which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement, and handling to manufacturing, distribution, and consumption of the finished product. Monitoring usually relies on surveillance of physical and/or chemical parameters such as time and temperature of heating or pH, whereas validation of HACCP performance requires testing for the absence of specific food-related pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes*, and others. There is some controversy as to whether microbiological tests can be used to monitor critical control points (CCPs) because of the length of the time needed to generate results and the sampling strategy required to obtain meaningful results. However, considerable advantages may accrue if microbiological control can be performed quickly and implemented into HACCP system.

The problem of rapid microbiological analysis for food and environmental samples is further complicated by necessary sensitivity (“zero tolerance”), and by the fact that bacteria in both environmental and food samples are not distributed evenly most of the time, resulting in statistically sound sampling requirements. According to requirements of food inspection agencies worldwide, “zero-bacteria” in food and/or environmental samples mean that there is not a single cell of target pathogens in 25 g of sample. In practice, for most nonliquid materials, 25 g of the substance is homogenized in 225 ml of media (or buffer) and is analyzed as a whole. Therefore, the required sensitivity is 1 cell per 250 ml of sample. Currently, there is no method available that is capable of reaching such levels of sensitivity without an extended enrichment step. Two methods that are currently the closest to
a real-time format for detection are real-time polymerase chain reaction (real-time PCR) and ATP-bioluminescence.

For RT PCR the term “real-time” does not actually mean that detection of bacteria occurs in real time. Instead, this method allows each cycle of DNA amplification to be observed on the computer screen during the sequence of thermal cycling, hence the designation “real-time.” In general RT-PCR method comprises the following major steps:

(1) enrichment and/or separation/concentration: 6 to 48 h;  
(2) DNA preparation: about one hour;  
(3) RT PCR reaction: 0.5 to 1.5 h.

Detection of a single cell of pathogen in 25 g of food by PCR requires, on average, 1–3 days, which is much faster than any available immunoassay (3–5 days), but is still far away from a real-time format.¹

Bioluminescent microbiological assays are based on the detection of intracellular adenosine-5-triphosphate (ATP) released from the target bacteria, using bioluminescent enzymatic reaction catalyzed by firefly luciferase. Theoretically this method allows for the detection of a single bacterium in the sample. Due to the fact that ATP is present in all live cells, the specificity of an ATP-based assay for certain pathogens relies on the preliminary separation and concentration of the specific bacteria. The following steps are included in the regular experimental protocol:

(1) enrichment and separation of target cells and removal of exogenous ATP (5 min–24 h);  
(2) release of intracellular ATP (1–2 min); and  
(3) measurement of bioluminescence (1–2 min).

Both real-time PCR and ATP bioluminescence methods have potential for developing rapid microbiological assays on their basis. What is the advantage of ATP BL over RT PCR and why? The most time-consuming step in both assays is the cell separation and concentration. Thus, providing the development of fast and reliable techniques for bacterial cell separation/concentration from large volume samples, the bioluminescent tests can be preferable since it can be performed within single minutes, it is cheaper to run compared with real-time PCR, as well as it can be easily adapted to high-throughput format.

A key objective of this book is to provide readers with information on the current status of bioluminescent assays in microbiology. Both theoretical and practical aspects of the tests are discussed; and strengths and weaknesses of particular techniques are reviewed. Specific experimental protocols are presented to provide a user-friendly reference guide for a wide range of applications. However, bioluminescence techniques have so many various applications in microbiological assays that it is almost impossible to cover all of them in a single book. Rather than
describing every single experimental protocol available, general practical consider-
rations of existing methods are provided to help readers develop or improve their
own protocols.

The number of companies that produce instruments and kits for bioluminescent
analysis has increased dramatically during the last decade. The main area of indus-
trial bioluminescence application is hygiene monitoring for food processing plants
and establishments. These applications allow for fast and reliable monitoring of the
cleanliness of surfaces and equipment. There is no need for expensive complicated
equipment and highly trained personnel. These methods can be performed in
“field” conditions, using hand-held devices. More specific bioluminescent meth-
ods for the detection of certain pathogens in food and environmental samples
were developed recently. Most of them are not yet available commercially. It is in-
tended through this book to encourage those in need of rapid, specific, and sensitive
pathogen tests to become familiar with novel bioluminescent techniques and imple-
ment them in the routine diagnostic and control systems.

The book has three main sections. The first deals with the basics of bio-
luminescence. In this section, general mechanisms of bioluminescent reactions are
discussed and major bioluminescent systems most relevant to analytical applica-
tions are reviewed. The second part of this book describes principles of biolu-
minescent analysis and their use for bacteria detection. The third section provides
experimental protocols available for bioluminescent detection of bacteria in differ-
ent food and environmental samples.

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