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Review

Recent applications of hyperspectral imaging in microbiology

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ABSTRACT

Hyperspectral chemical imaging (HSI) is a broad term encompassing spatially resolved spectral data obtained through a variety of modalities (e.g. Raman scattering, Fourier transform infrared microscopy, fluorescence and near-infrared chemical imaging). It goes beyond the capabilities of conventional imaging and spectroscopy by obtaining spatially resolved spectra from objects at spatial resolutions varying from the level of single cells up to macroscopic objects (e.g. foods). In tandem with recent developments in instrumentation and sampling protocols, applications of HSI in microbiology have increased rapidly. This article gives a brief overview of the fundamentals of HSI and a comprehensive review of applications of HSI in microbiology over the past 10 years. Technical challenges and future perspectives for these techniques are also discussed.

Contents

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1. Introduction

Despite advances in sampling and the development of rapid and automated techniques in microbiology, alternative analytical techniques that can be implemented on-line or can provide results in shorter time frames have been or are currently being investigated, including computer vision, spectroscopic methods, molecular and electronic noses and DNA-based methods. The assurance of microbial safety is of utmost importance for many industries, not least the food industry. This is not only because products of high quality and safety are increasingly expected and demanded by the consumers, but also because food safety legislation is more and more stringent [1]. The need for accurate, fast and objective food inspection methods that ensure safe production throughout the entire production process continues to grow. Microbial safety assurance methods have traditionally involved sampling and classical microbiological analyses, which are laborious and time consuming, and do not offer immediate results.

Hyperspectral chemical imaging (HSI), also known as spectral imaging or imaging spectroscopy, goes beyond conventional imaging and spectroscopy through obtaining spatially resolved spectra from an object. Similar to conventional spectroscopic techniques, HSI can be carried out in reflectance, transmission, scattering, transflectance or fluorescence modes. However, the added spatial dimension enables the mapping of chemical components in a sample, which is useful for non-homogeneous samples. HSI has been applied in many diverse and applied areas of analytical chemistry, such as food, agriculture and pharmaceuticals. More recently, HSI modalities have been applied to microbiology. A wide range of studies have been carried out, from understanding chemical reactions within bacterial cells via Raman HSI through to predicting total viable counts on food surfaces using Near infrared HSI. It is thus timely to review the recent applications of HSI in microbiology. These applications can be grouped into three major categories: (1) fundamental papers where HSI is used to gain information on biochemical processes of relevance to microorganisms; (2) applied papers where HSI is used to detect and identify microorganisms of concern at a microscopic scale, usually grown on "artificial" substrates such as stainless steel, silver or selective agars; (3) applied papers where HSI is used to detect microorganisms of concern at a macroscopic scale, grown on "real" substrates such as food or typical food preparation surface (e.g. formica). Using these broad categories, the remainder of this paper is organized as follows: Section 2 provides an overview of typical instrumentation in HSI is presented, with special consideration of the challenges inherent to the application of HSI to microbial samples; Section 3 is a brief overview of techniques typically used to analyse data arising from HSI experiments; Section 4 provides an overview of the state of the art in the application of HSI to microbiology, including critical reviews of the literature; Section 5 summarises the limitations and future perspectives for HSI from a microbiologists perspective and this is followed by the main conclusions in Section 6.

2. Instrumentation

Hyperspectral chemical imaging techniques are available for most traditionally single point spectroscopic methods (e.g. flourescence, visible (Vis), infrared (IR), Fourier-transform IR (FTIR), near infrared (NIR), Raman spectroscopy), although the instrumentation required for each modality varies significantly. However, the typical core components of any HSI system are: light source, wavelength modulation system and detector.

2.1. Light sources

In Vis–NIR HSI systems, the sample/target is usually diffusely illuminated by a tungsten-halogen (TH) light source. These broadband sources cover a wide wavelength range, from 400 to 2500 nm and are low cost [2]. However, the heat generated by TH bulbs is significant and may alter or even burn the sample. This is an important consideration in imaging live microbial samples or foods. To overcome this issue, fiber optic line lights are often used to provide distance between the TH bulb and sample (an example is the set-up used in [3]). Many other types of light sources are also used such as light emitting diodes (LED) which can cover a wide spectral range, from the visible to the near-infrared region. LED light sources have some advantages with respect to TH bulbs due to their small sizes, long lifespan and prevention of sample heating. However, LEDs covering the NIR range are currently more expensive than TH sources and LEDs only provide narrow wavebands of light, making them more suitable for multi-spectral imaging. Vis–NIR hyperspectral fluorescence imaging, useful for identifying biofilms on foods, is typically carried out using UV-A (365 nm) lamps as light sources [4,5].

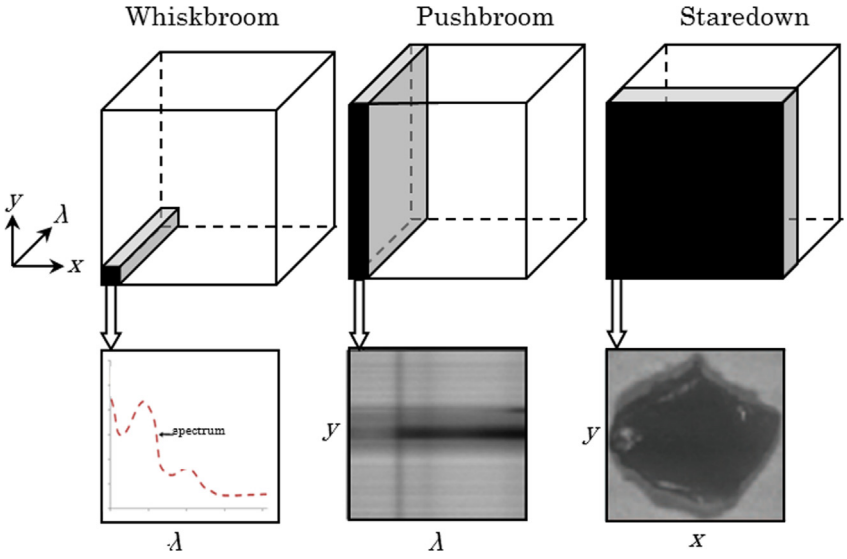

Fig. 1. Schematic showing three working modes of hyperspectral imaging systems taking imaging a chicken breast fillet as an example. Black areas in the upper row denote detected portion of a chicken sample in respective modes and the lower row shows corresponding obtained data during each scan.