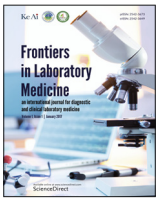
Frontiers in Laboratory Medicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Frontiers in Laboratory Medicine

journal homepage: www.keaipublishing.com/en/journals/frontiers-in-laboratory-medicine;



Label-free techniques for laboratory medicine applications

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ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form 4 May 2017
Accepted 19 May 2017
Available online xxxx

Keywords:
Label-free technologies
Terahertz radiation
Raman spectroscopy
Biochip/Microarray
Mass spectrometer

ABSTRACT

New technologies and advanced methods are the important basis for continuous development of laboratory medicine. Although the labeling analysis has become an important research method in laboratory medicine, label-free methods showed unique advantages, such as high sensitivity, small working volumes, low damage to analytes and easy on-chip integrations. Label-free methods are mainly based on the molecular biophysical properties without conjugated labels, which can largely avoid false positives and can provide more reliable and reproducible detection results. Nowadays, New-generation label-free technologies such as terahertz spectroscopy, Raman spectroscopy, Biochip/Microarray, mass spectrometer, have already shown great potential in biomedical applications, such as the identification of clinical microorganism, rapid and unlabeled detection of bimolecular, and dynamic monitoring of bimolecular interaction and the imaging of the construction and spatial structures. In this review, we summarized recent findings on the development and potential applications of the new-generation label-free technologies in clinical laboratory, focusing on the advantages and biomedical research of terahertz spectroscopy, Raman spectroscopy, Biochip/Microarray, quartz crystal microbalance devices and mass spectrometer.

Contents

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http://dx.doi.org/10.1016/j.flm.2017.06.003

New-generation label-free technologies such as terahertz spectroscopy enable high sensitivity analyzing capabilities with low sample consumption, low damage to analytes

Detection techniques have gone through tremendous innovative developments in recent decades. As early as 1959, Yalow and Berson combined radioactive elements labeling techniques with immunochemistry to analyze serum insulin for the first time. In the late 1970s and early 1980s, the radioactive elements were replaced by enzymes, fluorescent dyes, and luminescent agents. Nowadays, labeling techniques mainly includes fluorescent labeling, isotopic labeling, chemo-luminescent labeling, electrochemically active probe labeling, and Nanoparticle labeling.[1] The labeling analysis has become an important research method in laboratory medicine. However, the labeling process is inherently time-consuming, which can unavoidably alter the intrinsic properties of analytes. To solve the difficulties mentioned above, label-free techniques have been popularly studied. Based on the molecular biophysical properties (e.g., refractive index, molecular weight and molecular charge), label-free methods have unique advantages, such as high sensitivity, small working volumes, low damage to analytes and easy on-chip integrations.[2] Label-free techniques mainly include spectroscopy technology (e.g., Terahertz spectroscopy, Raman scattering spectra), biochip, quartz crystal microbalance (QCR), and mass spectrometry (MS).

Terahertz radiation provides an innovative label-free technique, possessing unique sensing ability with noninvasive and nonionizing properties

Terahertz radiation (THz, 1 THz = 1012 Hz) is kind of electromagnetic waves with frequency band from 0.1 to 10 THz. Terahertz radiation is a new type of far-infrared coherent radiation source, which falls in between infrared region and microwave region. In recent years, the biomedical application of THz technologies have developed a new discipline named terahertz biomedicine (THz-Biomed) with Terahertz laboratory medicine (THz-Labmed) as the core components of THz-Biomed.

THz-Biomed, or THz radiation, is proposed an efficient, label-free, reagent-less and nondestructive detection methods, which is indispensable for laboratory medicine. Studies show that low-frequency internal motions (e.g., skeleton vibration and rotation) and weak bonds (e.g., Hydrogen bond and van der Waals force) of biological molecules have a time scale which corresponds to THz radiation frequency range. Therefore, many biological molecules have specific THz fingerprint. THz spectroscopy has several unique advantages, compared with infrared spectroscopy, X-ray or other traditional spectral analysis techniques. Firstly, with low photon energy, THz wave is nonionizing for biological molecules, cells and tissues. So THz technology is particularly suitable for *in vivo* nondestructive examination. Secondly, with both imaging and spectroscopy properties, THz waves can detect not only the quantity and function of biomolecules, but also can analyze the composition and structure, which are difficult to be obtained by other electromagnetic bands. Thirdly, different biological molecules have specific THz fingerprint, thus THz radiation can simultaneously reveal the composition information, such as biological macromolecules, within clinical samples in a harmless way, which is indispensable for laboratory medicine.

The applications of THz technology in laboratory medicine will focus on the disease imaging diagnosis, label-free DNA sequencing, mechanism and absorption differences for biological tissue, bacterial rapid detection, interactions between biological samples, and the biosecurity on biological samples and biological processes. It has been reported that crystalline form of fructose and glucose can be identified by THz spectroscopy. And the characteristic THz spectrum fingerprints of several bimoleculars (e.g., peptides, nucleic acids, serum proteins, and collagen) have been gradually found.[3–6] For bacteria detection, Globus and co-workers obtained the unique THz vibration spectrum fingerprints of bacteria and achieved characterization of different bacteria.[7] Label-free quantification of DNA fragments were achieved by terahertz time-domain spectroscopy (THz-TDS). The detection limit was as low as 0.1 nag μL^{-1} and the minimum sample volume was only 10 μl.[8]

Besides, it is worth mention that a portable terahertz biomedical detector has been fabricated.[9] Moreover, terahertz detection system was developed for the detection of clinical tumor samples.[10] With the development of these hardware devices, THz technology has great potential in laboratory application.

Surface-enhanced Raman spectroscopy with ultra-sensitive capability and engineered selectivity has become a robust technique to analyze biomolecules

Raman scattering is a non-elastic scattering phenomenon, which is produced by wavelength changes of excited molecular. The energy difference between scattered light and excitation wave corresponds to the vibrational level of molecules, which allows Raman spectroscopy to be used for identification of particular chemical group. Surface-enhanced Raman spectroscopy (SERS), also known as surface-enhanced Raman scattering, is a type of vibration spectroscopic technique that enhances Raman scattering by molecules adsorbed on metallic nanostructures (e.g., metal nanoparticles, plasmonic-magnetic silica nanotubes). The Raman signal is largely amplified by roughened metal substrates with an enhancement factor as much as 10^{10}–10^{11}.[11,12]

SERS has become a promising candidate technique for various biomedical applications, and SERS based platforms are superior to traditional detection methods

Firstly, Raman scattering is almost instantaneous, which result in a more stable signal without photodegradation.[13] Secondly, kinds of SERS signal can be excited simultaneously by a single laser beam, which enable multiplexed detection of different targets. Thirdly, SERS signal has almost no water interference, which is particularly suitable for biological imaging. Furthermore, the required sample volume for SERS detection is extremely low and without pretreatment. Extensive efforts have been devoted to the application of SERS for the detection and imaging of bimoleculars (e.g., nucleic acid, protein, and cell). In recent years, noble-metal-free materials have exhibited superior performance, which overcome the shortcomings of noble-metal nanostructures based SERS platform. In 2008, stimulated Raman spectroscopy (SRS) has been developed. The SRS signal intensity has linear relationship with concentration without labeling and signal distortions. SRS has the inherent three-dimensional imaging capabilities, which describe the dynamic process of life activities through quantitative acquisition of images.[14] In 2011, Zhang et al. demonstrated imaging of nucleic acids and proteins in drosophila larvae salivary gland cells and mammalian cells, and real-time imaging of distribution changes of living DNA content was obtained, which contributed to a better understanding of cell development and proliferation process. In recent years, the Raman imaging technology showed potential to distinguish different types of breast cancer cells. SERS was used for the imaging of three kinds of molecules which are closely related to breast cancer.[16] This demonstrated that SERS has potential to be applied for the diagnosis of breast cancer.

Please cite this article in press as: Chen X., et al. Label-free techniques for laboratory medicine applications Frontiers in Laboratory Medicine (2017), http://dx.doi.org/10.1016/j.flm.2017.06.003