Modern optical techniques provide a bright outlook for cell analysis

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The states of the body go hand in hand with the physiological states of the cell, which are closely linked with each other through cell-related molecular structures and intermolecular interactions. Studies on cell analysis are important for the development of diagnostics and biomedical research.

Optical techniques reported in the literature include fluorescence, colorimetry, Raman scattering, surface-plasmon resonance and chemiluminescence, and those coupled with microfluidic systems.

This article reviews studies on the methodologies and recent developments in cell analysis by optical techniques between the beginning of 2008 and early 2012.

Keywords: Cell analysis; Chemiluminescence detection; Colorimetric bioassay; Fluorescent bioprobe; Fluorescence resonance-energy transfer (FRET); Label-free detection; Microfluidic system; Optical technique; Surface-enhanced Raman scattering (SERS); Surface-plasmon resonance (SPR)

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1. Introduction

The cell is the functional basic unit of life. Many biological systems have complicated architectures that are made up of cells (e.g., cells can synthesize new proteins, which are essential elements for modulation and maintenance of cellular activities and physiological functions of the body). Analysis of cell contents and characterization of cell behavior are essential to understanding aspects of cells (e.g., physiological state, differentiation, disease and metabolism) [1–3]. Also, cell analysis has proved to be an effective way to detection and to identify special or abnormal cells from the large number of cells, and could be used for early diagnosis of diseases. For detecting cell-based diseases, it is most important is to develop methods with high sensitivity at the cell level to collect small signals to be used for biological research and biomedical diagnosis. Analysts have developed techniques for this purpose.

Several optical-detection techniques designed for bioanalysis have sensitivity, wide dynamic range, and multiplexing capabilities. These techniques (e.g., micromanipulation) have the advantage over most traditional experimental approaches, as they cause less unwanted collateral damage and the probes can reach deep inside the specimens at the same time (provided the specimens are reasonably transparent).

This article reviews progress in chemical analysis of cells in the past three years, more or less split into components, such as cell detection and molecules in the cell membrane, intracellular molecules and cell-secretion analysis.

2. Fluorescence analysis and fluorescence imaging

Fluorescence has become a powerful tool in the field of cellular imaging and monitoring intracellular molecules. Fluorescent small molecules have been widely used for the visualization of biomolecules or cellular organelles and shown great promise for elucidating signaling pathways and performing cell analysis at the single-molecule level when these molecules are targeted to bind on the cell surface [4,5].

An imaging agent comprises one fluorophore moiety and one targeting functionality {e.g., antibody [6], peptide [7], DNA [8], or special ligand [9,10]}. In particular, the probability to detect a fluorophore signal from background depends upon the density of the fluorophore and therefore changes measure intensity distributions.

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Cells containing endogenous chromophores showing fluorescence exhibit cell autofluorescence. The well-known cell-autofluorescent chromophores are porphyrin, tryptophan, collagen, reduced nico tinamide adenine dinucleotide (NADH) and pigment [11,12]. The presence of noise and autofluorescent background – unavoidable in living cell recordings – influences measurements. Commonly used fluorescent dyes have significant limitations in sensitivity in applications, due to the interference from a high background noise caused by cell autofluorescence, which leads to poor photostability, strong photoblinking and overlap of emission peaks.

In recent years, due to the cell autofluorescence caused by ultraviolet excitation light, longer wavelength excitation has become desirable and several near-infrared (NIR) nanomaterials [13–15] have attracted much attention. Although these nanomaterials offer high photoluminescence efficiency, size-dependent emission wavelengths and sharp emission profile to facilitate relatively interference-free sensing, they are composed of heavy metals that possibly introduce cytotoxicity, which restricts their applications for cell-biorecognition events. Other new NIR fluorescent luminophores have therefore been made in the field of fluorescent bioprobes [16,17] (e.g., organic dyes and fluorescent proteins). Unfortunately, lifetime images are sometimes more important than intensity images, but the lifetimes of most organic fluorophores are close to that of cell autofluorescence which is 2–5 ns [18].

There therefore remain great challenges in developing high-performance fluorophores for cell analysis. Recent studies by the Yan group have demonstrated a photoactivated NIR fluorescent probe with an average fluorescence lifetime up to 85.6 ns, which was consistent with the improvement in the steady-state fluorescence [13]. Compared with traditional fluorescence biolabels, the new developments in NIR fluorescent probes have exhibited advantages in terms of being more easily isolated from the cell autofluorescence and more clearly distinguishable from each other in the lifetime cell images, which promise to be of great use in labeling biomolecules for bioanalysis.

Recently, some novel carbon-based nanomaterials (e.g., graphene and carbon dots) with inherent fluorescence properties in the NIR region proved interesting [19–22], while human tissues and biological fluids are practically transparent to these emissions [23,24]. Compared to fluorescent semiconductor nanocrystals, fluorescent carbon-based nanomaterials are superior in chemical inertness and eco-friendly properties, and potentially have low toxicity [25].

However, those carbon-based nanomaterials show insufficient fluorescence intensity, so it is not enough to meet the demand for sensitive cell analysis. But, their inherent quality of fluorescence quenching has attracted great attention. Graphene could be used as a fluorescence quencher and appeared to protect nucleic-acid probes from nuclease digestion or single-strand binding protein interaction, so it could be used effectively to transfer the nucleic-acid probe into cells for intracellular analytes [e.g., microRNA and adenosine triphosphate (ATP)], which would be promising in cell-based disease diagnoses [26,27] (Fig. 1).

Subsequently, green fluorescent protein (GFP) or its derivatives is commonly used as the reporter to quantify expressed protein levels due to its good biocompatibility. Nevertheless, it has intrinsic limitations (e.g., GFP maturation time, high background, and photobleaching) [28,29], so there is still much room for development of fluorescence probes to measure physical and biochemical changes of the cellular environment.

An extracellular supramolecular reticular DNA-nanoparticle (NP) sheath has been developed as a cage-type cellular probe for high-intensity fluorescence microscopy imaging (Figs. 2 and 3). The extracellular supramolecular reticular DNA-NP sheath is constructed from layer-by-layer self-assembly of DNA-NP probes and DNA nanowire frameworks functionalized with a Ramos cell aptamer. The DNA-NP sheath forms specifically and quickly on the surface of Ramos cells at physiological temperature, and produces exceedingly high fluorescence intensity. Using the extracellular supramolecular reticular DNA-NP sheath, Ramos cells can be clearly observed and easily distinguished from a mixture of multiple cancer cells by fluorescence microscopy imaging [30,31]. Based on the above research, various target cells were employed as proof-of-concept model for physical signs and metabolism using the fluorescence method.

There are several small molecules [32–40] (Figs. 4–7) and nanomaterials [41] as sensors of cellular biothiols (e.g., glutathione and cysteine). The molecular recognition steps of these approaches were based on interactions of the SH of the analyte and the probe molecules or nanomaterials through chemical reactions, which are not fast enough to allow real-time detection under physiological conditions. Besides, there was interference from cellular media (e.g., autofluorescence and light scattering).

To date, a number of detection methods have been described to alleviate these problems. Xi and co-workers [42] have developed a fast response fluorescence thiol-quantification probe by which the reaction was complete in less than 1 min at room temperature and can be used for real-time thiol quantification. Yan and co-workers [13] have designed a novel NIR fluorescent probe, effectively eliminating interference from autofluorescence and light scattering from cellular matrix, based on photoactivated CdTe/CdSe nanomaterials for detecting cellular biothiols. However, this method cannot avoid the toxic effects from cadmium.

There are also several fluorescence methods for intracellular pH (Figs. 8–10). Gao et al. [43] adopted