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Review

Potential of label-free detection in high-content-screening applications

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Abstract

The classical approach of high-content screening (HCS) is based on multiplexed, functional cell-based screening and combines several analytical technologies that have been used before separately to achieve a better level of automation (scale-up) and higher throughput. New HCS methods will help to overcome the bottlenecks, e.g. in the present development chain for lead structures for the pharmaceutical industry or during the identification and validation process of new biomarkers. In addition, there is a strong need in analytical and bioanalytical chemistry for functional high-content assays which can be provided by different hyphenated techniques. This review discusses the potential of a label-free optical biosensor based on reflectometric interference spectroscopy (RIfS) as a bridging technology for different HCS approaches. Technical requirements of RIfS are critically assessed by means of selected applications and compared to the performance characteristics of surface plasmon resonance (SPR) which is currently the leading technology in the area of label-free optical biosensors.

Keywords: High-content screening (HCS); Label-free optical biosensor; Reflectometric interference spectroscopy (RIfS); Cell-based assays

Contents

1. Introduction

The development of high-content-screening (HCS) assays seems to become the new driving force in drug discovery, and for selected diagnostic applications. The key issue of HCS is to overcome present restrictions, e.g. in the development chain for lead structures for the pharmaceutical industry or during the identification and validation process of new biomarkers. Therefore, in drug discovery the aim is the identification and validation of hits coming from the high-throughput screening (HTS) by applying HCS methods [1,2].

In its current understanding, HCS deals with multiplexed, functional cell-based screening technologies [3] and is mainly based on automated digital microscopy [4–6] and cytometry [7,8], linked to a fully automated experiment evaluation. But technological constrictions in combining analytical methods appear also during other complex bioanalytical processes

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because of the lack of "smart" assays suitable for high throughput with high specificity. This added value can be obtained, e.g. by new HCS assays combining separation sciences with determination of bioactivity and structure identification. Obviously, a new concept for high-content analysis based on hyphenated techniques is necessary. Hence, the general success of HCS is based on its integration in the highly developed systems for HTS. For example, automated electrophoresis offers the possibility to handle complex mixtures of biomolecules related to almost all fields in "omics" sciences. Here restrictions often occur during identification of interaction partners via functional bioassays.

In HTS, the aim of bioanalytics is the parallel detection of biomolecular interaction using either well plates or chip technology. Whereas fluorescence detection methods [9] using labeled compounds in various assay formats are now quite commonly used, direct optical detection without the support of labeled compounds is not as well established yet. For this reason, extensive research and technological development in the area of direct optical detection of biomolecular interaction have been carried out in recent years. Especially for the bioanalytical methods currently used in HCS one major disadvantage is the introduction of a fluorescence label. Here, label-free optical biosensors have already proven to be versatile tools without changing the natural properties of the investigated specimens [10]. Furthermore, direct optical biosensors for parallel detection are making use of modern imaging technologies [11]. Together with the ability of some techniques to measure living cells [12,13], a bridging technology from HTS to HCS is available.

Direct optical detection can be divided into methods that focus on measurement of changes in the refractive index of the interaction layer, and into methods based on changes in reflectivity at the layer. Regarding the first method, Biacore [14] has opened the market by introducing surface plasmon resonance (SPR) [15] as a very promising tool in biomolecular interaction analysis (BIA). Approximately 15 years ago, a very simple interferometric method has been developed, first as a detection principle in chromatography measuring changes in optical thickness as the product of refractive index n and physical thickness d [16,17]. A variation of this approach called reflectometric interference spectroscopy (RIfS) is now in use and known as a versatile label-free direct optical sensing method [10]. Compared to other label-free techniques, the potential of RIfS as a linking technique between HTS and HCS will be discussed. The intention of this article is to show that label-free detection with direct optical biosensors can contribute to the classical HCS as well as to the other upcoming high-content analysis problems.

2. Methodology

The label-free optical detection method for surface interactions, RIfS, is based on white light interference at transparent thin layers. At each interface of thin layers of different materials with negligible absorption radiation is partially reflected and transmitted. If the optical pathlength through these layers is smaller than the coherence wavelength, the different partial beams interfere, forming an interference pattern depending on the wavelength, the optical thickness which is given by the product of the refractive index of the layer and its physical thickness, the incident angle and, the refractive index of the surrounding medium [17,18].

RIfS uses the change in the optical properties in or at the top layer of a given layer system as detection principle (Fig. 1).

The binding of an analyte molecule or particle to the sensor surface causes a shift of the interference pattern in the wavelength domain. To evaluate the binding signal, the locus of an extremum is tracked over time; thus, the change of the interference spectrum results in a time-resolved binding curve of the analyte molecule to the sensor surface.

The RIfS standard transducer consists of a glass substrate (~1 mm thick) coated with a layer of a material with high refractive index (usually Ta_2O_5 or Nb_2O_5), and a top layer of SiO_2. For special applications other layer systems and substrates are used, e.g. PMMA (polymethylmethacrylate) or TOPAS (a cyclic olefin copolymer) [19]. When investigating interactions of biomolecules on surfaces, non-specific binding to the sensor must be minimized. Therefore, the glass slide is coated with a shielding layer which prevents non-specific binding and additionally provides a high number of functional groups for the immobilization of the binding partner. The standard shielding chemicals used are dextranes which form a three-dimensional hydrogel loaded with a high number of binding sites [20], and polyethylene glycols (PEG) forming a two-dimensional (2D) brush-like monolayer with less binding sites but with a more defined surface [21]. Amino and carboxy groups are normally functional groups for the immobilization process because the well-established peptide chemistry methods can be applied.

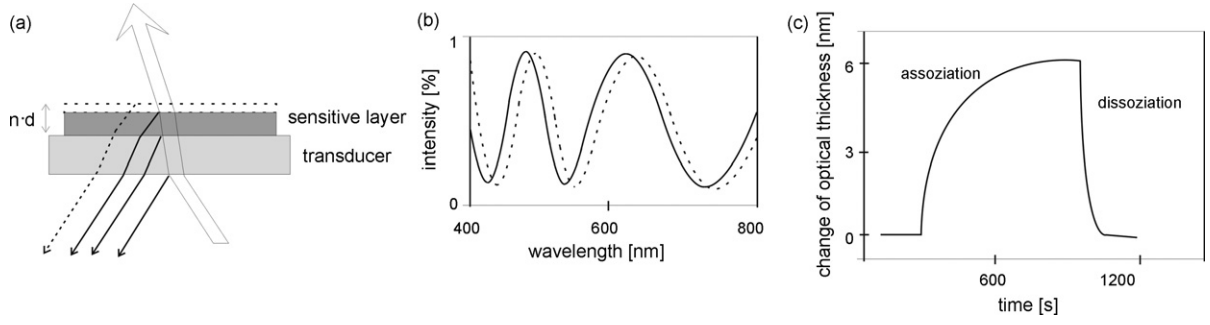

Fig. 1. Scheme of the RIfS detection principle. (a) Superimposition of the reflected light beams and the change of the optical thickness during a binding event on the sensor surface. The corresponding change of the characteristic interference spectrum is shown in (b) and the resulting binding curve is given in (c).