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# Label-free optical biosensor for on-line monitoring the integrated response of human B cells upon the engagement of stimulatory and inhibitory immune receptors

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ABSTRACT

In this study a novel, label-free method using the evanescent field based resonance waveguide grating technology was applied to examine B cell activation. We successfully immobilized non-adherent B cells on the surface of the biosensors, without the ligation of any specific receptors or adhesion molecules. This way we were able to demonstrate that engagement of the antigen specific B cell receptors (BCR) induced reproducible dynamic mass redistribution (DMR) inside the cells as a measure of receptor activation. The initiated DMR response proved to be specific, since only antibodies recognizing the BCR could generate the response; neither the assay-buffer, nor high concentration of indifferent proteins or non-specific antibodies had any effect. The measure of cell activation was sensitive, concentration dependent, and specifically and dose-dependently inhibited by the Syk inhibitor BAY 61-3606. The BCR-triggered DMR response was evoked from three human Epstein-Barr virus (EBV) negative B cell lines, but could not be elicited in two EBV-positive BL cell lines, where the presence of the EBV-derived LMP2A protein desensitizes the cells' response to the BCR-induced signaling. Parallel engagement of the inhibitory FcγRIIB together with the BCR resulted in the inhibition of the activation of B cells demonstrating that the DMR response mirrors the expected interaction between the downstream signaling events of the two receptors. As a multi-target profiling procedure, this label-free RWG technology is applicable for the study of complex cellular signaling via the analysis of integrated real time signature of DMR. Therefore, our work opens new avenues to study complex signaling events and to decipher interactions within the signaling network during B cell activation.



## 1. Introduction

Functional, cell based assays used in studying receptor ligand interactions add further versatility to those of biochemical binding methods; in addition to the sensitive and reliable monitoring of ligand binding, functional cell based assays permit the identification of ligands with properties which are not predictable from a single binding result. They allow the recognition of receptor agonist or antagonist compounds [1], but in addition inverse agonists or allosteric modulators as well [2,3]. Cellular assays are able to provide information about not only the fact but the biological consequences of a receptor ligand interaction and therefore their use is widely accepted and is on the rise. [4,5] The majority of current cell-based assays relies on the measurement of a single event at a predetermined time point in a specifically chosen signaling pathway, let it be second messenger release, reporter-gene production or target translocation [6]. These measurements require the use of labeled compounds, sometimes the modification of cells to express the target in larger amount or to produce a reporter molecule to be able to monitor receptor engagement. The mentioned manip-

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ulations can be toxic for the cells and can interfere with normal cellular physiology of the target receptors or their environment [7,8] and the applied fluorescence and colored compounds may induce elevated background [9,10]. Therefore the potential to identify false-positive responses increases with the application of these approaches. Consequently, functional cellular assays which can report from different signaling events in real time without the application of molecular engineering (in providing the suitable cellular partner) and without the use of labelling (in producing the ligand for the reaction) would be of high value for both theoretical and practical studies even if they are more complex and less specific than cell-based biochemical assays.

Satisfying these desires several label-free technologies and platforms have been developed during the past few years (reviewed in e.g.: Fang, 2011[4]; Scott and Peters, 2010[5]) using biosensors sensitive to changes in either the electric or optical properties of a thin layer enclosing certain portions of the indicator (proteins, cells) above the sensor surface. Among the optical sensors interferometry based waveguide configurations, dual polarization interferometry (DPI) and grating coupled interferometry (GCI), were employed for monitoring surface processes in aqueous solutions [11,12]. These methods are very sensitive but due to the special cuvette designs these are less ideal for monitoring living cells. They have only two independent sensing areas and without auto sampler they are low throughput methods [13]. Waveguide based techniques, offer higher sensitivity than the relatively more widespread surface plasmon resonance (SPR), typically employed to study biomolecular interactions in a label-free manner [14,15]. Among waveguide based techniques optical waveguide light mode spectroscopy (OWLS) [16] was already employed to monitor living cells but it lacks the necessary throughput for statistical analysis of the cellular responses [17]. The newly introduced miniaturized high throughput EPIC plate reader potentially could also be employed in signaling experiments of living cells [18], but its validation in this area has just been started. However one of the resonant waveguide grating (RWG) optical technologies (EPIC) [19] have received special attention recently due to its use as a pharmaceutical screening method, for monitoring modifications in G-protein coupled receptor (GPCR) related pharmacological responses [20–22] and for its potential to deconvolute complex GPCR signaling [23]. Completed and published studies – using the RWG optical biosensor – were not restricted to the above mentioned and other GPCR-related examinations. They included experiments with receptors having tyrosine kinase activity like the analysis of epidermal growth factor (EGF) receptor triggered reactions [24] or different cellular applications, such as investigations on general cellular behaviors [25], cellular adhesion [28] and ion-channel responses [27]. From these studies it has become clear, that the RWG technology provides non-invasive continuous real-time recording of cellular activity with high sensitivity. It is a useful tool in drug discovery both in the evaluation of compound efficacy or specificity and in the determination of its mode of action. With resonant waveguide grating technology the measured signal is an integrated cellular response that involves several successive and/or parallel signaling triggered cellular events, including cytoskeleton reorganization, and/or movement of signaling cascade proteins, which altogether result in the dynamic rearrangement of cellular mass content (Dynamic Mass Redistribution [DMR]). Therefore, RWG, together with other label-free technologies, is a multi-target profiling technique, which is applicable for the study of complex cell signaling via the integrated real time signature of DMR, and allows analyzing and deciphering so far unknown interactions within the signaling network.

B-lymphocytes recognize foreign (non-self) antigens in the body via their antigen-specific receptor (B-cell receptor, BCR), and they mount antibody response upon the encounter. It has been established for a while that during the development and control of a successful humoral immune response not only the BCR mediated triggering, but the balanced signaling via immune complex binding complement and Fcγ-receptors is also necessary [28]. B cells respond to these stimuli and depending on the final tuning and integration of the different instructions coming from the various receptors, their response could be activation, survival and differentiation [29–31], changes in antigen uptake [32] inhibition of the response [33,34], or unresponsiveness [35,36], depending on the developmental stage and the micro environment of the cells [37–39]. Mature B-cells can be activated via their BCR by foreign materials (antigens) entering the body, and during an ongoing immune response antigen antibody complexes are formed. These immune complexes (IC) can be recognized by Fc-receptors also, bringing an additional level of complexity to the developed response [40]. These and other (e.g. contact with complement components and receptors) interactions could dramatically influence the outcome of the activation of the competent cells involved; and via these they modulate the development of an effective humoral immune response. Clarification of the particulars and especially identification of the integrated consequences of these interactions might help us to identify possible points for therapeutical intervention. To be able to obtain holistic pictures about B-cell responses to complex interlocking stimulations, we decided to apply the Epic BT optical biosensor, and set to establishing the method using human B cell lines, derived from Burkitt's lymphomas.

## 2. Materials and methods

### 2.1. The Epic® BenchTop resonant waveguide grating imager biosensor

The Epic® BenchTop (BT) system (Corning Incorporated, Corning, NY, USA) is a next-generation resonant waveguide grating (RWG) imager biosensor allowing high-throughput label-free detection at a solid liquid interface using evanescent optical waves [41,26]. The sensing principle of RWGs is very similar to the well-reported working principle of Optical Waveguide Lightmode Spectroscopy (OWLS) [16,17]. Similarly, a nanostructured grating area is employed to excite the waveguide mode of a planar optical waveguides. But, in RWGs the grating is designed such a way that after a short distance of propagation (in the micrometer scale), the waveguide mode is out-coupled into the direction of the substrate. This feature makes RWGs ideal for sensor array developments, since the optical signals of neighboring sensing units are not disturbing each other. Moreover, all of the optics can be placed below the sensor array and liquid handling can be performed over the sensing surfaces in a straightforward manner. The RWG imager employed in the present study accepts 96- or 384-well *Society for Biomolecular Screening* (SBS) standard format biosensor microplates. In the present work, a 384-well uncoated cell assay Epic® microplate (Corning, 5040) was used. The bottom of the microplate is a planar optical waveguide – a thin, high refractive-index, transparent dielectric layer (waveguide layer, made of the biocompatible material niobium pentoxide) deposited on a thicker glass substrate. At the central position of each well, a $2 \times 2$ mm optical grating is embedded into the optical structure to enable the interrogation of the zero$^{th}$ order transverse magnetic ($TM_0$) waveguide mode using near-infrared electromagnetic radiation. Thus individually addressable biosensors are created in each well of the microplate. All wells of the Epic® microplate are simultaneously interrogated every 3 seconds by sweeping the illuminating wavelength through a range of 15 nm with 0.25 pm precision (in the range of 825–840 nm). Waveguide mode excitation only happens at a resonant wavelength ($\lambda$). Refractive index variation in