

Live Cell Optical Sensing for High Throughput Applications

Ye Fang

Abstract Live cell optical sensing employs label-free optical biosensors to non-invasively measure stimulus-induced dynamic mass redistribution (DMR) in live cells within the sensing volume of the biosensor. The resultant DMR signal is an integrated cellular response, and reflects cell signaling mediated through the cellular target(s) with which the stimulus intervenes. This article describes the uses of live cell optical sensing for probing cell biology and ligand pharmacology, with an emphasis of resonant waveguide grating biosensor cellular assays for high throughput applications.

Keywords Cell-based assay • Cell signaling • Dynamic mass redistribution • G protein-coupled receptor • High throughput screening • Optical biosensor • Resonant waveguide grating biosensor

Contents

- 1 Introduction
 - 2 High Throughput Biosensor Systems
 - 3 Live Cell Optical Sensing Detects an Integrated Cellular Response
 - 4 Live Cell Optical Sensing for Receptor Biology
 - 5 Live Cell Optical Sensing for Ligand Pharmacology
 - 6 Live Cell Optical Sensing for High Throughput Screening
 - 7 Conclusion
- Reference

Y. Fang
Biochemical Department, Science and Technology Division,
Corning Incorporated, Corning, New York 14831
e-mail: fangy2@corning.com

Abbreviations

β_2 AR	β_2 Adrenergic receptor
GPCR	G protein-coupled receptor
H ₁ R	Histamine receptor subtype 1
HTS	High throughput screening
RWG	Resonant waveguide grating
SPR	Surface plasmon resonance

1 Introduction

Cell-based assays that examine the activities of live cells have become the powerhouse in both cell biology and drug discovery in the past decades, due to their ability to facilitate the measurements of mode of action, pathway activation, toxicity, and phenotypic cellular responses of exogenous stimuli. However, most cell-based assays suffer from two drawbacks. First, these assays typically measure “point-of-contacts”, such as the generation of a second messenger, the translocation of a tagged protein, or the expression of a reporter gene, in a myriad of cellular events downstream ligand-induced receptor activation [1]. Thus, the activity of a ligand obtained is only related to the specific cellular event measured, but does not reflect the full potentials of the ligand acting on the live cell or cell systems [2, 3]. Second, these assays require certain manipulations of cellular contexts or background (e.g., over-expression of targets with or without a readout tag); and such manipulations could result in false information of receptor biology [4, 5].

Optical biosensors including surface plasmon resonance (SPR), resonant waveguide grating (RWG) and plasmon-waveguide resonance are routinely used for biomolecular interaction analysis [5, 6]. These biosensors are label-free and are capable of measuring minute changes in local index of refraction at/near the biosensor surface. Recently, we [7–13] and others [14, 15] had applied these biosensors for live cell sensing. We have found that these biosensors enable monitoring endogenous receptor activation in real time, leading to high-information and physiologically relevant measures of a receptor–ligand pair [see reviews 7, 8]. These assays do not require prior knowledge of cell signaling, and are pathway-unbiased [9, 10]. Instead of measuring single point-of-contacts within complex signaling cascades that most cellular assays do, the live cell optical sensing relies on an integrated cellular response relating to dynamic mass redistribution (DMR) to probe receptor biology and ligand pharmacology [11–13]. This article reviews the recent advances in high throughput instrumentation, and theoretical considerations and applications of live cell optical sensing.

2 High Throughput Biosensor Systems

RWG belongs to a family of optical waveguide-based biosensors, and uses the resonant coupling of light into a waveguide by means of a diffraction grating (Fig. 1). A broadband and polarized light is used to illuminate the waveguide; light of a specific wavelength (the resonant wavelength) at which a maximal incoupling efficiency is achieved is coupled into and propagate along the waveguide [16]. The resonant light eventually leaks out the waveguide film and reflects back. The change in resonant wavelength is in proportion to change in local mass density at the sensor surface.

A significant advantage of RWG over conventional SPR is that given appropriate biosensor design, lights at nominally normal incident angle can be used to illuminate the biosensor [17]. This is important for sampling large numbers of biosensors simultaneously, which is a prerequisite for high throughput (HT) screening. Assaying samples in a typical HTS often directly takes place in the Society for Biomolecular Sciences standard microtiter plates, such as 384well microplates. The Epic[®] system from Corning Inc is the first system that is suitable for both biochemical- and cell-based assays in high throughput. This system consists of a RWG detector, an external liquid handling accessory and a scheduler, such that it can process large numbers of microplates using kinetic measures for high information content screening [10], or using end-point measures for HT screening [17].

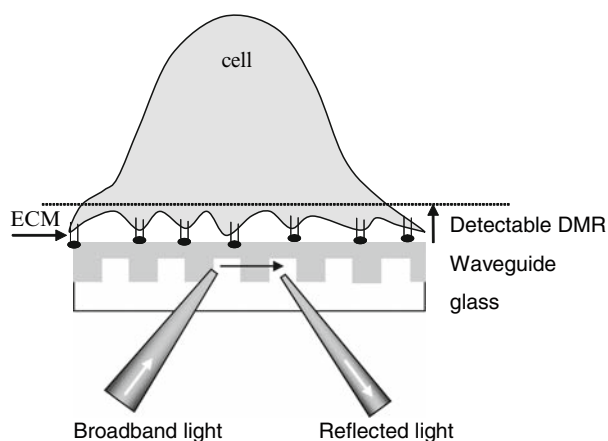


Fig. 1 Principle of live cell RWG sensing. Cells are brought to contact with a biosensor surface through interaction with the extracellular matrix (ECM).

3 Live Cell Optical Sensing Detects an Integrated Cellular Response

Live cell optical sensing generally exploits a surface bound evanescent wave [16, 18] to characterize stimulus-induced cellular responses [9–14]. For RWG biosensors, the evanescent wave is an electromagnetic field created by the total internal reflection of resonant light in the waveguide thin film, whose intensity exponentially decays away from the sensor surface. The distance from the sensor surface at which the electromagnetic field strength has decreased to $1/e$ of its initial value is the penetration depth or sensing volume (typically $\sim 150\text{nm}$). Considering the large dimension of live cells, a live cell RWG sensing system can be viewed as a three-layer waveguide: a substrate with an optical grating, a high index of refraction waveguide coating, and a cell layer. Since the local index of refraction within a cell is a function of density and its distribution of biomass (e.g., proteins, molecular complexes) [19], a ligand-induced change in effective refractive index (i.e., the detected signal) is believed to be governed by [13]:

$$\Delta N = S(C)\alpha d \sum_i \Delta C_i(t) \left[e^{\frac{-z_i}{\Delta Z_c}} - e^{\frac{-z_{+H}}{\Delta Z_c}} \right],$$

where $S(C)$ is the sensitivity of the biosensor to the cell layer, ΔZ_c the penetration depth into the cell layer, α the specific refraction increment (about $0.18/\text{mL/g}$ for proteins), $\Delta C_i(t)$ the change in local concentration of biomolecules at the given location at a specific time, z_i the distance where the mass redistribution occurs, and d an imaginary thickness of a slice within the cell layer. This theory predicates, as confirmed by experimental studies [9–13], that the biosensor is capable of non-invasively monitoring cell signaling in real time, a stimulus-induced shift in resonant wavelength is dominated by the DMR perpendicular to the sensor surface, and the resultant DMR signal is an integrated cellular response containing contributions from many cellular events mediated through a receptor.

4 Live Cell Optical Sensing for Receptor Biology

Live cell optical sensing has found broad applications in cell biology, including cell signaling [7–14, 20, 21]. Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. Cell signaling is encoded by spatial gradients and temporal modifications of cellular molecules upon receiving an exogenous signal [22–24]. The temporal modifications of key proteins in the posttranslational state, enzymatic activity, or total level can act as molecular signals that are relayed and interpreted to control cell function [25, 26]. Furthermore, the spatial targeting of signaling molecules and assemblies to appropriate sites, coupled with its temporal dynamics, is crucial to regulate the

specificity and efficiency of protein–protein interactions, and to spatially separate protein activation and deactivation mechanisms, thus enabling the precise control of the amplitude, duration and kinetics of cell signaling [27]. As a result, there is often dynamic relocation of cellular matters within the cells, leading to a dynamic, directional and directed mass redistribution. A DMR when occurring in the sensing volume of a biosensor can be detected non-invasively by a label-free optical biosensor [11, 12].

Because of the integrative nature of DMR responses, live cell optical sensing is able to examine the complexity of receptor biology. An example is the signaling of endogenous bradykinin B_2 receptor in a human skin cancerous cell line A431. Bradykinin, a vasoactive nonapeptide, is implicated in the regulation of many physiological and pathophysiological responses, including vascular permeability, inflammation, pain, and neurotransmitter release [28]. We found, using live cell optical sensing, that the signaling of B_2 receptor in A431 is dependent on cellular contexts [12, 17]. In proliferating A431 cells, low doses (<100nM) of bradykinin trigger G_s -mediated signaling, while high doses (>100nM) of bradykinin favors G_q -signaling. On the other hand, bradykinin between 0.5nM and about 100nM mediated dual signaling – G_s and G_q -pathways – in partially quiescent A431 cells obtained through continuous culturing with 0.1% fetal bovine serum for about 20hours (Fig. 2) [12]. We also found that in the partially quiescent cells the bradykinin response is specific to B_2 receptor activation, and is sensitive to the integrity of lipid rafts. Using chemical biology approach which exploits chemical intervention to probe receptor biology, not only critical downstream cascades

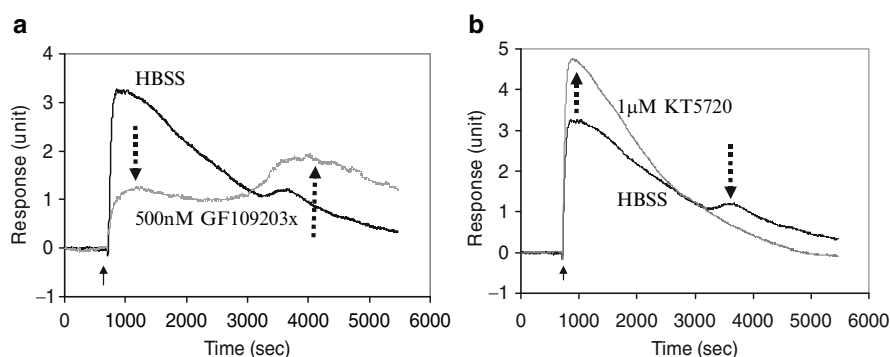


Fig. 2 Dual signaling of bradykinin B_2 receptor in A431 cells. The bradykinin-induced DMR responses of cells pre-treated with the “vehicle” (HBSS) were in comparison with those pretreated with 500nM GF109203x (a protein kinase C inhibitor) (a), or 1 μ M KT5720 (a protein kinase A inhibitor) (b). The bradykinin concentration was 16nM. The solid arrows indicate the time when the agonist solution is introduced. 1 unit in the y-axis equals to 100 pm shift in resonant wavelength. Chemical modulation showed that in the quiescent cells blockage of protein kinase C activity partially attenuates the early response but potentiates the late response (a), while blockage of protein kinase A activity potentiates the early response but attenuates the late response (b). These results suggest that the activation of B_2 receptors leads to both G_q and G_s signaling, and the two signaling pathways counter-regulate each other. Data was reproduced with permission from [12].

including protein kinase C, protein kinase A, and epidermal growth factor receptor, but also several cellular processes including endocytosis and cytoskeleton modulation had been found to be involved in B_2 signaling. These findings are in great agreement with previous studies using conventional cell biology approaches, which showed that the B_2 receptor in A431 can be mitogenic or anti-mitogenic, depending on the cellular states and the doses of bradykinin [29, 30].

5 Live Cell Optical Sensing for Ligand Pharmacology

Ligand pharmacology can be complicated. This is partly due to the intrinsic property of a ligand that often displays off-target effects and/or pathway-biased efficacies [2, 31], and partly due to the complexity of receptor signaling [32–36]. The pathway-biased efficacy is also referred to ligand-directed functional selectivity. A classic example is the G protein-coupled receptor (GPCR) signaling. A GPCR may couple simultaneously to more than one G protein subtype, and interact with other signaling molecules such as arrestins [33, 34]. In many cases the activation of a receptor can mediate both G protein-dependent and independent signaling, often in a ligand-dependent manner [35, 36]. As a result, GPCRs display rich behaviors in cells [37], and many ligands can induce operative bias to favor specific portions of the cell machinery and exhibit pathway-biased efficacies [31, 2]. It is obviously difficult in practice for conventional and pathway-biased cellular assays to systematically determine the signaling capacity of GPCR ligands.

Since live cell optical sensing is a highly sensitive functional assay and is applicable to wide arrays of targets and cellular processes [7–14], it can be used to study the systems cell pharmacology including ligand-directed functional selectivity of a drug molecule acting on cells. The high sensitivity enables detecting weak biological signals such as those provided by ligands of low efficacy and/or affinity. The pathway unbiased nature, coupled with the real time kinetics, allows the direct detection of possible pathway-biased activity of ligands.

Figure 3 shows the structure, DMR signatures and parameters of four agonists acting through endogenous β_2 AR in A431 using live cell RWG sensing. The four agonists differ greatly in their abilities to activate the β_2 AR, thus triggering signaling [38, 39]. The functional selectivity of agonists examined is clearly evident in their DMR characteristics. Interestingly, multi-parameter analysis uncovers distinct DMR patterns linking the structure to activities of these ligands. All four agonists led to similar responses but with great difference in fine features. Both the amplitudes of P- (positive-) and N- (negative-) DMR events reflect the efficacy of ligands. The full agonist epinephrine led to the greatest P- and N-DMR events. The occurrence of N-DMR is believed to be an indicator for the ability of the agonists to elevate intracellular cAMP level. The very weak partial agonist catechol was unable to produce any N-DMR event at all. The P-DMR kinetics, as shown by its $t_{1/2}$, is believed to be an effective indicator for ligands to induce receptor internalization. Both dopamine and catechol that are ineffective in causing receptor internalization led to faster P-DMR, compared to epinephrine or norepinephrine. Similarly, the transition time τ for the

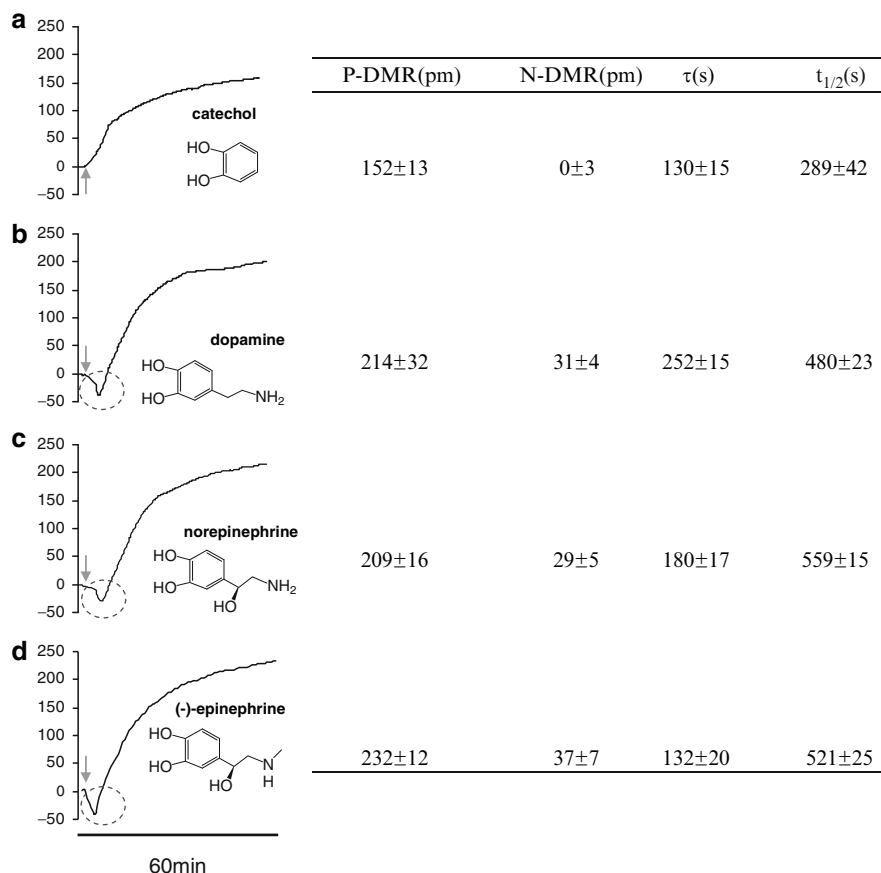


Fig. 3 The structures of β_2 AR ligands, and their DMR signals and parameters in quiescent A431 cells. The ligands included the full agonist (-)-epinephrine (8nM), the strong partial agonist norepinephrine (100nM), the partial agonist dopamine (32 μ M), and the very weak partial agonist catechol (500 μ M). The grey arrows indicated the time when the agonist is introduced. The y-axis is the response in picometer (pm). Data was reproduced with permission from [10].

P-DMR to occur is also agonist-dependent. These results illustrate the power of live cell optical sensing for probing ligand-directed functional selectivity.

6 Live Cell Optical Sensing for High Throughput Screening

HT screening that quickly screen massive numbers of compounds is crucial to drug discovery and development, due to the increasing size of compound library and the increasing numbers of druggable targets. Live cell optical sensing holds great potentials in HTS. Unlike conventional assays that often lead to potential false negatives due to the pathway biased nature, as well as high false positives due to

labels or cell engineering, live cell optical sensing offers a non-invasive and manipulation-free alternative to assay endogenous cellular targets such as GPCRs in native cells. Additional advantages of live cell optical sensing for HTS include the ability of multiplexing [1, 7], and of multi-modes [40].

An end-point HTS assay using Epic® system has been carried out for the endogenous β_2 -adrenergic receptor (β_2 AR) in A431. Results showed that the time to assay a single 384well plate is short (~5min per plate), and the assay is quite robust with a Z' of greater than 0.7 [17]. This study has stimulated high interests in industrial companies for potential HTS applications. Several successful HTS campaigns using live cell optical sensing have been performed recently by pharmaceutical companies.

We have recently developed a duplexed label-free optical biosensor cellular assay for simultaneously assaying two endogenous receptors, the G_i -coupled histamine receptor (H_1 R) and the G_s -coupled β_2 AR, in A431 cells [40]. Coupled with both agonism and antagonism modes in a single kinetic assay, we have shown that the agonist screening not only identified all full agonists for both H_1 - and β_2 -receptors, but also detected pathway-biased ligands for the β_2 AR (Fig. 4). Furthermore, the succeeding antagonist screening documented all known antagonists in the library for either H_1 or β_2 receptors. This is the first demonstration of a single cellular assay that is capable of screening ligands against two GPCRs coupled to distinct G proteins, and highlights the power of pathway-unbiased and label-free biosensor cellular assays for GPCR screens.

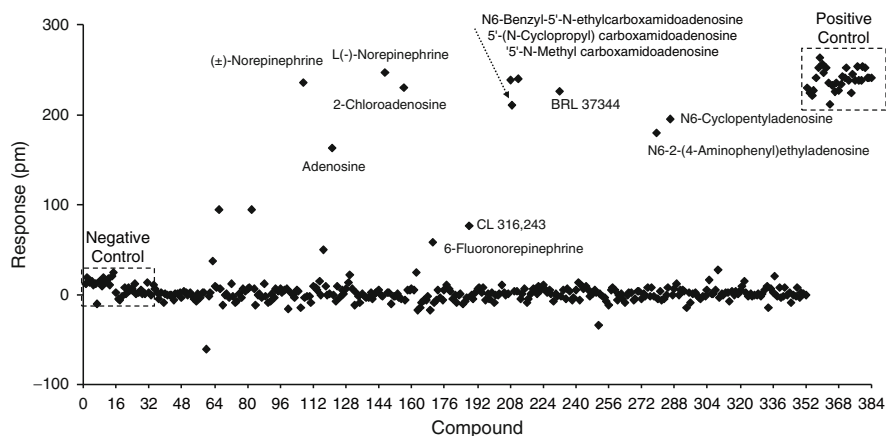


Fig. 4 An example of duplexed receptor screening in a 384well microplate format. The responses of quiescent A431 cells, in the initial agonism mode, were plotted as a function of compound. The quiescent A431 cells were stimulated with a subset of LOPAC library of compounds. The shift in resonant wavelength before and 50min after stimulation was calculated for each well. The negative controls (the “vehicle”) led to a response of 8 ± 7 pm ($n = 32$), while the positive controls (2nM (–)-epinephrine) led to a response of 230 ± 12 pm ($n = 32$), indicating a robust assay with a Z' factor of 0.74. This end-point screening identified all agonists in this subset of compounds for both the endogenous β_2 AR and adenosine receptors in A431. Several other compounds whose mechanisms to trigger the DMR responses are unknown were also identified. Data was reproduced with permission from [40]

7 Conclusion

The drug discovery process has been constantly evolving from affinity- and targeted-based screens to systems biology- or biological or clinical activity-based screens. The advent of optical biosensors, particularly high throughput instruments and cell-based assays, will accelerate acceptance of optical biosensors in many points at many stages of the drug discovery process, for which both high information content and high throughput are important.

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