Solvent Correction versus In-line Reference Measurement

- Optical systems such as SPR and BLI often have difficulty with nonspecific noise when measuring low molecular weight analyte in organic solvents.
- Solvent correction is a type of reference measurement that can subtract this noise but requires carefully-prepared reference samples, a dedicated reference cell, and supplementary measurements, costing time and resources.
- FEB does not require additional solvent correction measurements because it is an electrical technique, not an optical one. The biosensor responds minimally to organic solvents, which are neutral particles. FEB uses an in-line reference measurement that reduces time to data and conserves sample.

ABSTRACT

Understanding the nature of biomolecular interactions is critical to successful drug discovery. Surface plasmon resonance (SPR) and Biolayer Interferometry (BLI) are common tools for investigating target and analyte binding due to their sensitivity to biomolecular interactions observed in real-time.[1,2] However, when including organic solvents to improve the solubility of small molecules, the solvents themselves produce a response orders of magnitude greater than that of the small molecules under investigation. Solvent correction can compensate for nonspecific noise in the data during post-processing, but preparing the reference samples often requires a dedicated sensor cell and consumption of precious biomaterial.[3]

The use of organic solvents with Field Effect Biosensing (FEB) technology yields sensor responses orders of magnitude lower than the sensor response of small molecule interactions, and a high signal to noise is achieved without the use of solvent correction. FEB performs in-line reference measurements on the same biosensor cell with experimental measurements which saves expensive reagents and reduces surface preparation and tool run time.[4]

INTRODUCTION

How Do Biomolecular Assays Work?

To better understand the need for reference subtraction measurements, it is necessary to first describe SPR and BLI.

Surface Plasmon Resonance

Surface plasmon resonance (SPR) is a common optical label-free technique for detecting interactions in real-time.[5] An SPR tool employs a sensor chip consisting of a glass surface coated with a thin layer of gold (as shown in Figure 1). The gold layer is covalently coupled to a layer of targets using any one of several well-known coupling chemistries. Polarized laser light is directed toward the sensor chip at a fixed angle, travels through the glass, and is reflected back from the gold surface to a detector. However, below a certain critical angle, the light will be totally internally reflected. When totally internally reflected, the laser energy is transformed into an evanescent wave in the gold layer mediated by surface plasmons, and the reflected light intensity away from the gold surface drops significantly. The exact angle at which this occurs depends entirely on the optical properties of the functionalized gold

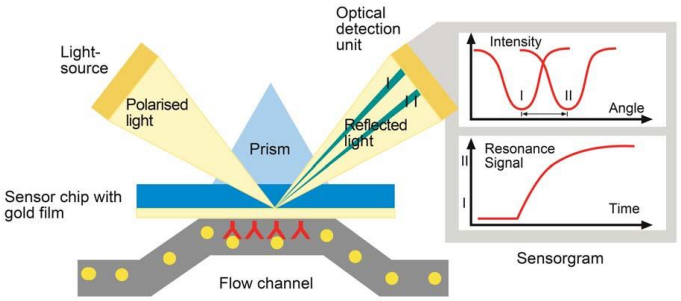

Figure 1: Schematic representation of a surface plasmon resonance tool. Polarized laser light is incident on a gold film which translates to an evanescent wave in the gold. The precise critical angle at which the light becomes redirected into the film is dependent on the biology on the sensor chip. The measured shift in the critical angle is recorded in real-time and translated into a sensorgram. From Reference 6.

surface. Binding of the analyte by the immobilized target changes the local index of refraction, which in turn shifts the critical angle for total internal reflection by a small but measurable amount by the detector. This shift in critical angle is continuously recorded in real-time and translated into response units as the analyte is loaded onto the sensor chip.

BioLayer Interferometry

BioLayer Interferometry (BLI) is another label-free technique for detecting protein and small molecule interactions.[7] BLI is also an optical technique whereby white light is sent down a fiber optic cable to interact with immobilized biomolecules (as shown in Figure 2). White light consists of a spectrum of wavelengths in the visible range. This spectrum of light can interact with both an embedded reference layer (i.e. the sensor surface) and the biolayer (i.e. the target) at the end of the fiber

optic cable before reflecting back into the fiber optic cable. The light collected back in the fiber optic cable is the result of interference between the reflected waves from the embedded reference layer and from the biolayer. When small changes occur in the biolayer, such as the binding of a analyte to its target, the spectrum of light interacts differently with the biolayer, traveling a longer distance before being reflected into the fiber optic cable. The interference pattern between the reflected wave without bound analyte is shifted relative to the interference pattern without analyte binding, and this shift is directly related to the amount of analyte bound. With state-of-the-art optical detectors and signal processing, the wavelength shift in interference pattern can be accurately monitored and translated to a binding curve in real-time where the magnitude of the wavelength shift becomes the sensor response.

Why Use Reference Measurements?

In all biomolecular assays, unintended signal can be recorded from sources other than the specific target and analyte pair being investigated. Undesirable sensor responses can come from slight differences between the running buffer (buffer without analyte) and the assay buffer (buffer with analyte) in terms of temperature, ionic strength, or solvent concentrations or from nonspecific binding of targets or analyte to the sensor surface. These effects are compensated for with reference measurements.

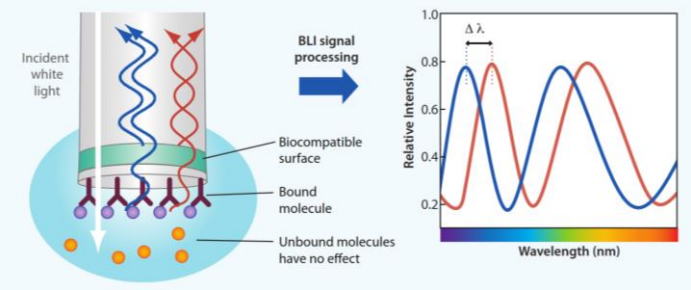

Figure 2: Schematic representation of a biolayer interferometry tool. Incident white light through a fiber optic cable is reflected back into the fiber optic cable from an embedded reference layer and from the biolayer attached to the end of the fiber optic cable. Peaks in the visible spectrum from each reflected wave are shifted as the analyte binds in real-time. The wavelength shift is translated to a sensorgram. From Reference 8.

Whether using solvent correction or in-line reference measurement, it is important to match the preparation of the reference samples with the experimental samples as closely as possible.[8,9] Differences in target density between the reference measurement and the experimental measurement can cause mass transport effects and displaced volume effects. It is possible to correct for these artifacts by subjecting each condition to a control solution and creating a calibration plot. Even under the most controlled conditions, a reference cell and its experimental counterpart can experience bulk and drift effects. Appropriate and error-free reference measurements are necessary to have faith in the sensing data.

What Is Solvent Correction?

Solvent correction, a type of reference subtraction measurement, is a method to subtract solvent effects from the total response of an optical system-derived sensorgram.[9] The index of refraction of a liquid can change significantly with minor changes in its composition. A light wave interacting with the solvent-containing liquid will be affected and induce a systematic noise response that may be larger than the effect of the analyte molecules. For example, a liquid containing the solvent DMSO (dimethyl sulfoxide) demonstrates a bulk SPR response orders of magnitude larger than the response from a small molecule (response to 1% DMSO on an SPR tool is roughly 1200 resonance units [RU] where a typical small molecule response is on the order of 10 RU).[10] Small variations as low as 0.01% in DMSO content lead to quantifiable variations in the bulk response between samples that can bury the analyte response, making meticulous sample prep vital for accurate small molecule characterization on an optical system.

Solvent correction protocols have been developed to better control for these effects through mathematical analysis.[11] The solvent in question is diluted to several known concentrations and injected into two adjacent cells: a blank reference cell with no target immobilized and an active cell with target bound. For each solvent dilution, the response of the reference cell is recorded as well as the response difference between the active and the reference cell. Once all solvent dilutions have been tested, the response difference of the active cell minus the reference cell is plotted against the response of the reference cell to determine the fit parameters describing this relationship. During the experimental measurement with analyte, the reference cell and the active cell are again measured in parallel. When the solvent interacts with the reference cell and induces a sensor response, the fit parameters determined previously will translate that signal into an appropriate error correction factor for the active cell, hence accounting for the effects of the solvent.

A more concrete example of solvent correction protocols is given in Figure 3. To begin, a blank reference cell and an active cell with immobilized target are prepared and exposed to multiple concentrations of the solvent-containing buffer without any analyte present (Figure 3a). The series of buffers should contain the organic solvent in concentrations bracketing the approximate concentration in the analyte sample, e.g. dilutions of 4 - 6% DMSO in steps of 0.2% if the analyte is in 5% DMSO. For each concentration, there will be a bulk response in each cell, which means two data curves per concentration, corresponding to the reference cell and the active cell (red and black curves in Figure 3b). From these data, the reference cell response is subtracted from the active cell response and

plotted against the reference cell response with each concentration of DMSO producing one data point on the plot (Figure 3c). The plot should be roughly linear, but second order effects will often complicate this step making solvent correction very difficult. The plot represents the effect of the organic solvent on the target itself as a function of the concentration of the organic solvent.

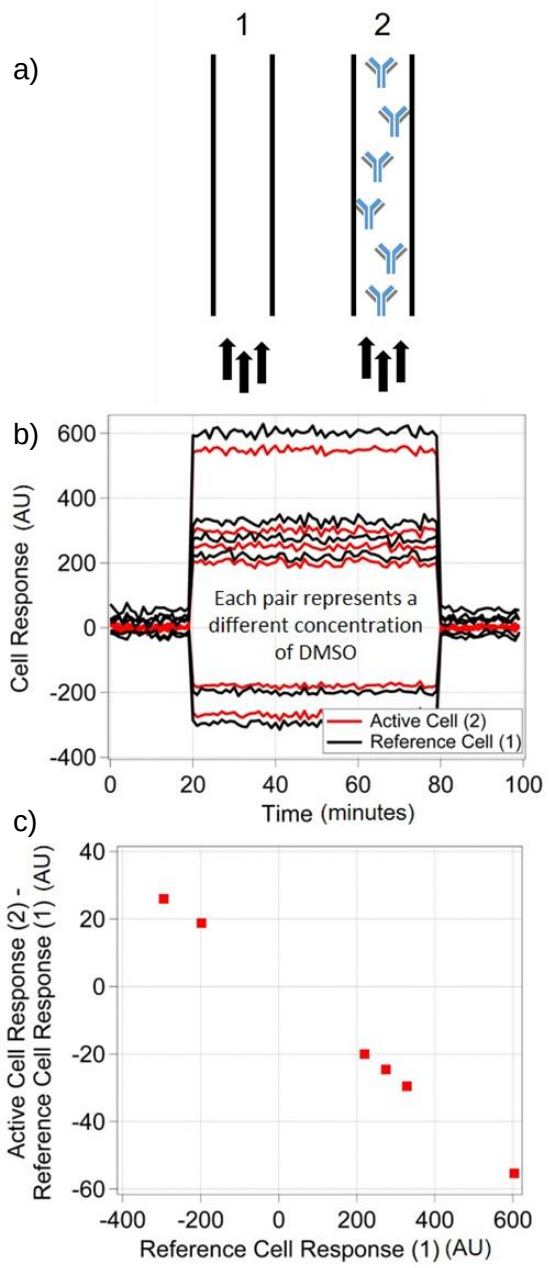

Figure 3: a) The reference cell and active cell being exposed to several sequential concentrations of organic solvent in buffer. (1) is the blank reference cell, and (2) is the active cell that has been functionalized with target. b) Each cell responds to various concentrations of organic solvent such as DMSO. Responses are different between (1) and (2) due to excluded volume and bulk effects. c) Response (2) minus Response (1) plotted against Response (1) extracted from the data in (b). This linear trend describes the net effect of the organic solvent molecule on the target as a function of organic concentration.

How Does Solvent Correction Affect My Sensor Response?

After the plot from Figure 3c has been generated, the analyte in assay buffer containing the organic solvent is injected into both the reference cell and active cell. To reiterate, the method behind the solvent correction procedure is necessary because the organic solvent can alter the immobilized target resulting in false signal, and the solvent itself can induce false sensor responses larger than those of the compound being investigated. If the reference subtraction measurement is performed with analyte in 4.98% DMSO and the experiment is performed with analyte in 5.02% DMSO, that small variation in DMSO concentration is enough to prohibit accurate data collection.

Running the assay buffer with analyte through both cells will induce responses (Figure 4a). The response of the reference cells is plotted on the x-axis of the graph from Figure 3c to extrapolate the corresponding effect of the DMSO on the active cell. In this example, the reference cell recorded a bulk sensor response of ~ -140 arbitrary units (AU) (Figure 4b), which corresponds to an active cell response of 12.78 AU larger (Figure 4c, new black dot plotted against red dots from Figure 3c). This value is the amount to be corrected due to solvent response on the active cell.

When analyte is in solution, the response of the active cell will contain the target binding its analyte convoluted with the sensor response due to the organic solvent. Any systematic linear offset due to solvent can be corrected easily enough with standard referencing (simply subtracting the reference cell response from the active cell response). However, the sensor response from organic solvents such as DMSO is variable over time, so solvent correction (double referencing) entails correcting the measured exponential fit curve that represents the small molecule response to compensate for the 12.78 AU response from the organic solvent interacting with the target. The data are corrected and fit to a new exponential with the saturation response decreased by the amount determined from the previous solvent correction step, in this example 12.78 AU (Figure 4d).

Solvent correction is a complex multi-step process rife with opportunities for error. However, it is critical to perform this process when the expected analyte responses are low, the target is densely bound to the measurement surface, and the absolute bulk response to the assay buffer is high and subject to variation between sample solutions.

Do I Need to Perform Solvent Correction on My Tool?

Solvent correction is required for optical tools like SPR and BLI tools since the organic solvents in the sample change the index of refraction and affect the sensor response significantly. When interrogating the sample with light of any kind, whether white

light or a laser, the optical response is perturbed by the solvent, and the effects of the solvents must be corrected to obtain accurate results.

FEB Does Not Require Solvent Correction

Optical systems are significantly affected by organic solvents like DMSO because of the modality of the sensing measurement. DMSO shifts the index of refraction of the solution, so any technique relying on measuring index of refraction changes will be swamped by organic solvent signal.

However, electrical sensing techniques can measure small molecules in organic solvents without the use of solvent correction since they do not rely on measuring changes to the index of refraction. FEB measures local changes in the electrostatic profile to register biomolecule binding events in real-time (Figure 5). Since organic solvents are net neutral molecules, they do not significantly affect the sensor response and do not require solvent correction (Figure 6). Further, small molecules yield large sensor responses with FEB due to their large charge to mass ratio, and the sensor response magnitude is large for small molecules.

What Are the Benefits of FEB?

With FEB, the slight variation in organic solvent concentration does not affect the sensor response or mask the detection signal. In-line reference measurements can be accomplished on the same sensor cell used for experimental measurement by adding assay buffer without analyte. It is not necessary to dedicate an entire cell to a reference measurement: all sensor cells can be used to collect data on experimental samples. Expensive or difficult to synthesize biomolecules are not being wasted on extra reference cells, thus preserving precious material. With all referencing occurring in minutes instead of hours, unstable molecules with short half-lives can be fully characterized using FEB where they might otherwise denature during lengthy solvent correction runs on optical sensors. Without the need for solvent correction, FEB enables measurement of small molecules in a shorter time frame with lower consumption of precious biomolecule material without relying on software algorithms to reveal the biology.

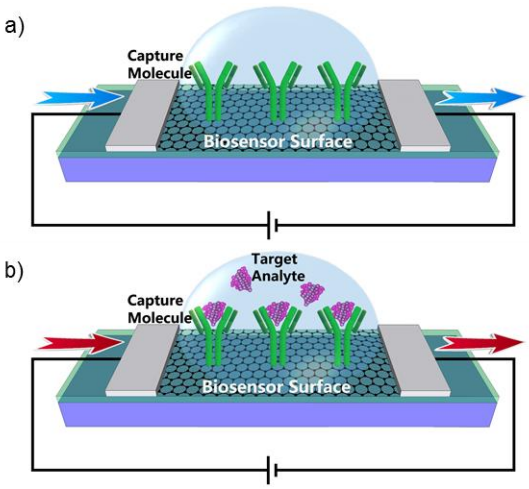

Figure 5: An illustration of FEB. The electrical current through a nanomaterial cell is altered when a analyte binds to its corresponding target at the sensor surface. The magnitude of the shifts in electrical properties directly correlate to the concentration of bound analyte and can be measured accurately in real-time.

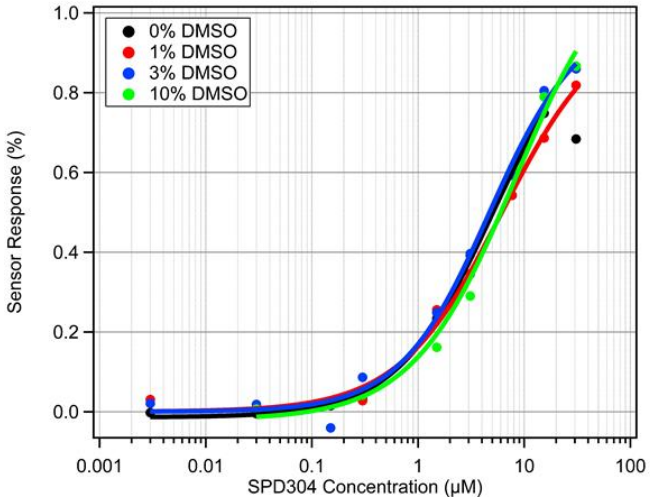

Figure 6: Standard curve from an FEB tool (Agile R100 biosensor chip from Nanomedical Diagnostics) functionalized with TNFα, a cell signaling protein implicated in Alzheimer's disease and cancer. The analyte is SPD304, a small molecule inhibitor of TNFα. Each colored set of data points represents a concentration series of SPD304 in a buffer containing 0%, 1%, 3%, or 10% DMSO. The results indicate that sensor response is unaffected by DMSO concentration and does not require multi-step referencing protocols. Small molecules yield a large sensor response on FEB due to their high charge to mass ratio.

CONCLUSIONS

FEB provides an alternative route to investigating small molecule binding kinetics in buffers containing organic solvents without the need for solvent correction, a dedicated blank reference cell, or complex data processing. Targets in limited supply or with a short lifespan are particularly well-suited to be characterized using FEB. In-line reference measurements are sufficient to correct for systematic effects in the data, reducing the tool run time and providing a more stable and straightforward user experience.

REFERENCES

1. Nirschl M, Reuter F, Vörös J. Review of transducer principles for label-free biomolecular interaction analysis. *Biosensors*. 2011;1(3):70-92. doi:10.3390/bios1030070.
2. Cooper MA. Optical biosensors: where next and how soon? *Drug Discov Today*. 2006;11(23-24):1061-1067. doi:10.1016/j.drudis.2006.10.003.
3. Masson JF, Battaglia TM, Cramer J, Beaudoin S, Sierks M, Booksh KS. Reduction of nonspecific protein binding on surface plasmon resonance biosensors. *Anal Bioanal Chem*. 2006;386(7-8):1951-1959. doi:10.1007/s00216-006-0834-2.
4. Lerner MB, Pan D, Gao Y, et al. Large scale commercial fabrication of high quality graphene-based assays for biomolecule detection. *Sensors Actuators, B Chem*. 2016:1-7. doi:10.1016/j.snb.2016.09.137.
5. McDonnell JM. Surface plasmon resonance: Towards an understanding of the mechanisms of biological molecular recognition. *Curr Opin Chem Biol*. 2001;5(5):572-577. doi:10.1016/S1367-5931(00)00251-9.
6. Dahms N. The BIAcore 3000 Theory, Applications & Techniques.
7. Concepcion J, Witte K, Wartchow C, et al. Label-free detection of biomolecular interactions using BioLayer interferometry for kinetic characterization. *Comb Chem High Throughput Screen*. 2009;12(8):791-800. doi:10.2174/138620709789104915.
8. Tobias R, Kumaraswamy S. *Biomolecular Binding Kinetics Assays on the Octet Platform*. Menlo Park, CA; 2013.
9. *Biacore™ X100 Handbook*. Uppsala, Sweden; 2008.
10. GE Healthcare Life Sciences. Biacore Assay Handbook. *GE Healthc Bio-Sciences AB*. 2012:1-78.
11. Cooper MA, Karlsson R. Experimental Design. In: *Label-Free Biosensors: Techniques and Applications*. Cambridge University Press; 2009:38-39.

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