Abstract

Measurement of molecular interactions is essential for fundamental biological studies as well as for the development of health diagnostics devices. Several label-free and labeled techniques to characterize and quantify molecular interactions have been developed. A major disadvantage of the labeled techniques is the requirement of an additional process step of labeling that increases the system complexity for completely automated systems. There are many label free molecular detections techniques such as electrical (nano wires and nano tubes based), mechanical (cantilever and quartz crystal micro balance based), electrochemical and optical (interferometry, waveguides, optical cavities and surface plasmon resonance (SPR) based). The requirements of an ideal bio-molecular sensing technique are a) cost effective, portable and simple to operate, b) high sensitivity and specificity, c) high reproducibility, d) real time measurement capability and e) ability to do parallel measurements in a Lab-on-Chip format.

The goal of the work done in this thesis is to design an optical real time bio molecular sensing technique that should be cost effective and highly sensitive. In this work we describe the use of self-referenced measurement techniques to eliminate common mode signal drifts due to thermal and concentration gradients in the fluid flow cell and demonstrate a nearly 40-fold decrease in the system noise. It started with a reflectance modulation point by point scanning system. Polyelectrolytes (poly allylamine hydrochloride (PAH) and poly acrylic acid (PAA), negatively and positively charged polymers) microarray was created on a SiO2 grown silicon substrate. Reflectance modulation was analyzed from the SiO2 surface and polyelectrolyte microarray on the SiO2. But the limit of detection (LoD) was poor due to laser intensity fluctuations.

Therefore, a self-referencing diffractive reflectance modulation system (DRMS) was proposed. There, diffractive microstructures were fabricated in the SiO2 layer on top of silicon substrate and created a flow cell on the diffractive microstructures. When these diffractive microstructures are illuminated by a laser beam, they produce a diffraction pattern in reflected and transmitted light. In the diffraction pattern the zeroth contains average background information whereas higher orders contain signal information of the liquid sample present on the microstructures. By using Si/SiO2 devices we demonstrated bulk (refractive index sensing for water ethanol mixtures) and surface adsorption (real time binding of polymer layers on micro structured Si/SiO2 devices) molecular detection measurements. Instead of doing bio molecular detection measurements on Si/SiO2 devices we started doing measurements on glass devices with the advantages, flexibility to do measurement in transmission and lower substrate cost.

In the next part of the thesis, bulk and surface adsorption measurements were demonstrated for glass substrates. For this, a 2D periodic array of wells of 10-micron diameter was created in the glass cover slide with various well depths. A flow cell was constructed on the diffractive micro structured cover slide to inject liquid samples on the microstructures, and this micro patterned glass cover slide was used as our sensor device for bulk and surface detection measurements. With the glass sensor devices bulk and surface adsorption bio molecular detection measurements were demonstrated both in transmission and reflection. To demonstrate the bulk detection, we measured the refractive index of different

concentrations of NaCl in water and for bulk measurements a limit of detection (LoD) of 3x10-6 RIU was achieved. For surface adsorption measurements, layer by layer (LBL) deposition of positively (PAH) and negatively (PAA) charged polymers was demonstrated at the glass micro array sensor surface. The experimental data were validated with the model developed using transfer matrix method in chapter 4. Bio-molecular detection measurements were not demonstrated with this technique because this technique is in very preliminary stage of development. This technique needs more work to be done for standardization and optimization of appropriate conditions of detection protocols for biological samples. Large number of trials are necessary to standardize this technique under various control conditions because detection of proteins and DNA samples will add a whole new dimension of complexities depending upon size, coverage, and orientation of biological samples.

The same diffractive self-referencing concept can be applied for SPR also. For this, grating structures available on commercial DVDs were used for the excitation of surface plasmons. On these DVD samples, metal dielectric multi layers were coated and demonstrated to couple surface plasmons and give multiple SPR peaks in transmission as a function of incidence angle. By using diffractive self-referencing concept an intensity referenced SPR method was demonstrated for bio molecular detection measurements. With the intensity referenced SPR as a proof of concept for bulk measurements refractive index detection of different concentration of NaCl in water was demonstrated. Similarly, for surface adsorption measurements, polyelectrolytes PAH and PAA were used for the functionalization of surface and detection of Bovine Serum Albumin (BSA) was demonstrated. Only very preliminary experiments were done to demonstrate that the ratio metric self-referencing concept can be exploited in grating coupled SPR also for
bulk and surface bio-molecular detection measurements. This technique also needs many trials and repetitions for the standardization and optimization for use with biological samples.

A self-referencing method was introduced and demonstrated, and this method was applied to two different optical label-free sensing techniques, namely, interferometric reflectance modulation and SPR. As a proof of concept for bulk detection, real time refractive index measurements were done for different concentrations of ethanol and NaCl in water and for surface adsorption detection, real time layer-by-layer (LBL) assembly of polyelectrolytes PAH and PAA was demonstrated on Si/SiO2 and glass micro array sensor surfaces. These real time self-referencing techniques namely DRMS and intensity referenced SPR need lot more work to be done to realize their true potential for bio-molecular detection measurements. The performance of these techniques was studied from the point of view of simplicity, cost effectiveness, sensitivity, and robustness.