Abstract

The aim of this work was to develop methods for designing high-throughput ellipsometric biosensors. Today, this type of sensor is widely used in medical diagnostics. The acquisition principle of these methods is based on measuring thickness variations caused by binding of a species from solution to a second species on the sensor surface. From the time dependent data, information on binding rates can be obtained. As this method is noninvasive, the reactivity of biomolecules is unaffected. A high demand lies on methods that allow for screening large amounts of substances in parallel. These methods are named high-throughput screening (HTS). HTS of multiple samples requires sophisticated techniques respective sample application and measurement. As ellipsometric measurements are sensitive to thickness and refractive index, special care must be taken in producing homogenous sensors, applying sample substances in form of monolayers and in providing precise measuring tools. Deviations lead to a variation in sensitivity between different measurement sites. To overcome these issues, a sensor device allowing for addressing and optimizing the sensitivity of each site would be of high value, resulting in comparative data of all events in one run.

The first part of this work was dedicated to investigations on sensitivity issues caused by the measurement setup and sample application.

Using surface plasmon sensors, a special technique, „aligned soft lithography“, has been developed enabling to print arrays of different substances on sensors. The substances are applied evenly in form of monolayers by subsequent printing with different stamps. Such patterned sensors can be read out using imaging ellipsometry. Measurement routines provided with ellipsometers can be distinguished in region of interest (ROI) -based measurements and mapping routines. Both methods have issues, with ROI-based measuring of multiple sites being not reliable and imprecise, and mapping being precise but very slow which hinders from measuring fast kinetics. An improved method based on Off-null ellipsometry has been developed that enables to image reactions providing high acquisition speed and precision. Sharp images of the entire field of view can be acquired and stored for

evaluation by image processing. Time-dependent data are obtained in form of image sequences. From these images, thickness maps can be calculated facilitating fast evaluation of a large amount of reactions. In addition to that, the ellipsometric values used with Off-null ellipsometry provide a better performance than the values obtained from Null ellipsometry. This aids in measuring inhomogenous and thick species. By acquiring reactions with substances in form of arrays, it was possible to derive sensitivity issues caused by the measurement setup. The parameter with the largest contribution to differences in sensitivity between single sites was the illumination beam used with imaging ellipsometry. Imaging of large areas requires expanded light beams being not perfectly collimated. This causes the single sites being measured under different angles of incidence which influences the sensitivity.

The second part of this work was dedicated to developing a method that aids in optimizing the sensitivity of all measurement sites. The class of waveguide sensors was found to be sensitive to events in the reaction medium and to changes in the substrate. This can be utilized to design a device that can be modified in its response, respective the sensitivity.

A measure to facilitate this was found by introducing a photochromic „command layer“ that can be manipulated using light. This element comprises a photochromic protein, namely bacteriorhodopsin (BR) or proteorhodopsin (PR), being immobilized in a sol-gel glass of the ormosil type. A novel method was developed that allows to embed the sensitive proteins into this material by carefully optimizing the sol-gel process. The obtained material was of high optical quality and stability. It is demonstrated, that the response of waveguide sensors deposited onto this photochromic layer could be influenced by using light. Illuminating these devices with light spatially modulated in intensity allowed to pattern the response which could be observed in a lateral modulation of the ellipsometric images. The effect of the modulation was equal to the sensitivity deviation caused by illuminating the sample with a diverging measurement beam. This allows for equalizing the sensitivity of the sensor. Furthermore, it was shown that the effect can be used to compensate for variations in signal caused by refractive index changes in the analyte solution. This is important to maintain sensitivity while introducing reagents into the measurement cell.