

IR - Synchrotron Mapping Ellipsometry for Characterisation of Biomolecular Films

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In previous synchrotron studies nanostructured and biomolecular films have been characterized by IR ellipsometry [1-5] and complementary methods (VUV ellipsometry, XRD and AFM) with respect to the thin film structure. Recently in cooperation with Sentech Instruments the IR synchrotron mapping ellipsometer was upgraded with a rotating retarder option (see Fig. 1). This rotating retarder delivers a continuous change of the introduced phase shift without changing the optical path of the reflected radiation. This additional phase shift e.g. allows more sensitive measurements when $\cos\Delta$ is close to one. Previously for this purpose a prism in ATR geometry was used, which afforded a change of the optical path.

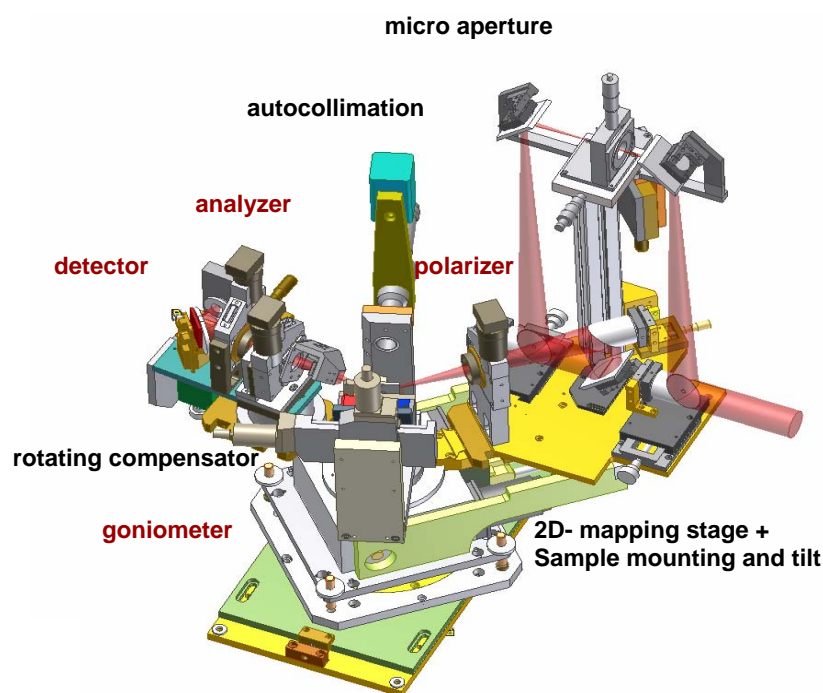


Fig. 1: Infrared (IR)-Mapping ellipsometer with 2-dimensional mapping stage, autocollimation, rotating retarder and microfocus unit at the IR synchrotron beamline at BESSY in Berlin.

In the following the first results taken with the upgraded IR synchrotron mapping ellipsometer are shown.

A) Biosensors

Currently used standard methods to read out microarrays are dependent on fluorescence and chemiluminescence-imaging techniques. These methods require labeling of a component by a marker and usually only detect the concentration of the marker molecule. A label-free imaging method that also enables a quantitative spectroscopic analysis of the composition and, moreover, possible interaction of components would be of great advantage. Therefore IR mapping ellipsometry (Fig. 1) was applied for the label-free imaging of a biochip before and

after incubation with peptide solution [3]. The general principle of such biosensors is based on the fact that peptides, proteins, enzymes, antibodies, antigens and DNA-fragments can be attached via specific linker molecules to a silicon chip. Electronic recognition of the specific adsorption site can serve for fast analysis of the samples under investigation. Our measurements show that IR ellipsometry is a sensitive tool for laterally resolved identification of the different materials and determination of the composition of a biosensor. The biosensor has been prepared by photo-electrochemical deposition of the linker (TFPAM-3 (*N*-(4-azido-2,3,5,6-tetrafluorobenzyl)-3-maleimidopropionamide)) on a hydrogen passivated silicon surface. After that, part of the linker covered wafer was incubated with peptide (Cys-GCN4) solution. Ellipsometric spectra of the biosensor were taken before and after incubation with the peptide solution. Fig. 2 shows maps (6 mm x 6 mm) of amplitudes of a linker specific band at 1288 cm^{-1} and a peptide specific band at 1547 cm^{-1} . From both maps linker and peptide covered regions of a sample surface are easily identified. As expected, the peptide was only found on those sample regions which were initially incubated by peptide solution, while the non-modified “linker regions” do not show the characteristic peptide band at 1547 cm^{-1} . Under certain assumptions (isotropic film, thickness independent structure of the films) the band amplitudes of a thin film directly correlate with the thickness of the film. Presently the qualitative interpretation of the band shapes is in agreement with an isotropic film structure. However, future work will be directed onto a more comprehensive evaluation of the experimental spectra by optical simulations.

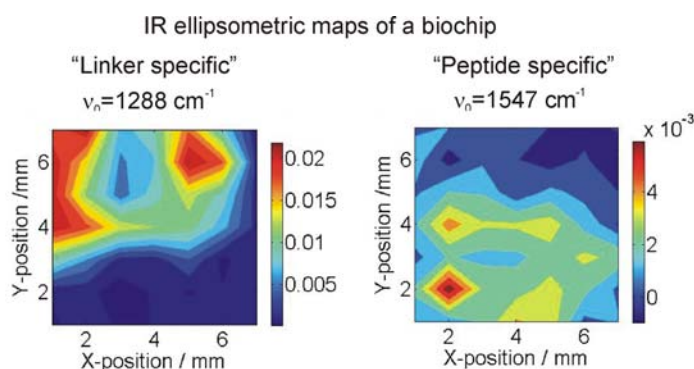


Fig. 2: Infrared ellipsometric maps of a biochip: $\tan \Psi$ band amplitudes of a linker specific band at 1288 cm^{-1} and a peptide specific band at 1547 cm^{-1} for a 6 mm x 6 mm area [3].

B) Thin cytosine film

In order to test the upgraded ellipsometer with rotating retarder a 110 nm thin cytosine film has been measured over an area of 3x3 mm^2 . The probed spots had a size of approx. 1 mm^2 in this case. By reducing the micro aperture 250x250 μm^2 lateral resolution can be achieved. Fig. 3 shows the average of the single spot spectra of the 3x3 mm^2 area in comparison to the lab measurement of the same sample.

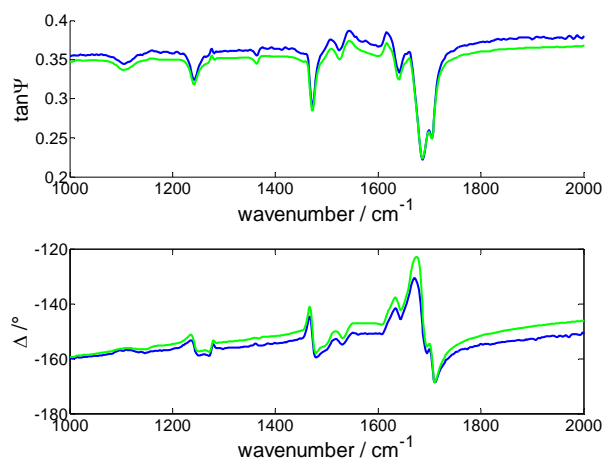


Fig. 3: Comparison of averaged synchrotron measurements (blue) of 1 mm^2 spots from a 3x3 mm^2 area and lab measurements (green) of a larger sample area ($\sim 7 \times 7 \text{ mm}^2$). The sample was 110 nm thick cytosine film on silicon.

A good agreement between lab and synchrotron measurements is found. However the spectra show slight deviations. This might be explained by the following reasons: 1) The high brilliance of the synchrotron radiation (e.g. smaller opening angle, less radiation is reflected from the backside of the transparent substrate on the detector); 2) selectively irradiated parts of not homogeneous optical elements (polarizer, detector) 3) in lab experiments the irradiated spot was larger than the sample area ($\sim 7 \times 7 \text{ mm}^2$), therefore also contributions of the inhomogeneous edges of the sample have to be considered in this case.

Fig. 4 shows maps of $\tan\Psi$ and Δ values at a frequency above the frequency range of vibrational bands of cytosine (at 1911 cm^{-1}). The maps prove that the prepared cytosine film is of homogeneous thickness. The maximum deviation of Δ is 0.2° .

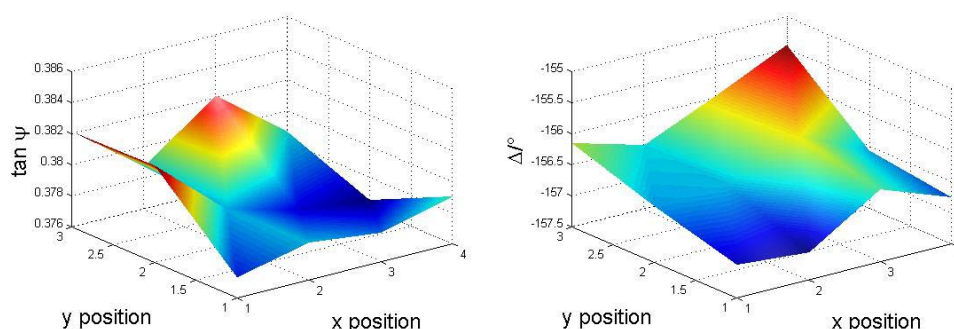


Fig. 4: Infrared ellipsometric maps of the thin cytosine film: absolute values of $\tan\Psi$ and Δ at 1911 cm^{-1} for an angle of incidence of 64° .

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