Characterization of glass micro array

Introduction

Commonly used quality control of DNA arrays employs hybridization, spotting of labeled DNA, and measurement of the Reflection of salt components left from the spotting buffers. Control hybridizations have the disadvantage that the slides used in the control cannot be reused. Spotting of labeled DNA does not guarantee that all other spots are found on the array. Control by measuring residual salt has the disadvantage that the DNA itself is not detected. By contrast to these methods imaging ellipsometry is the only method that allows the direct control of DNA arrays after the final (washing) step in a label-free and non-destructive manner. The images visualize not a 'carrier' component as salt but the DNA itself. Each spot is well resolved and can be judged regarding size, shape, homogeneity, positioning within the array. Imaging ellipsometry allows a quality control of DNA arrays without the need for control hybridizations or control spotting of labeled DNA. It measures thickness, refractive index, and (in case of labeling even) extinction of the spotted reaction layer.

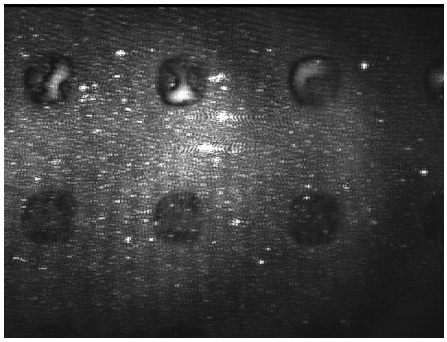

Fig. 1: Contrast image 0.8 mm x 1 mm

Sample

Typical Micro Array (spotted DNA reaction layer, some spots are fluorescence labeled, homogeneous activation layer, e.g. silan, glass substrate, e.g. BK7)

Instrumentation

Standard Imaging Ellipsometer EP³-SW (532 nm) or ArrayInspector (prototype), automatic sample handling stage, 5x-objective, EP³View Software Version 2.0

Task

1. Mapping and profiling of single spots or small clusters of spots
2. Measurement of thickness (or equivalent mass area density) of all spots

Steps of evaluation

1. Visualization of spots appr. 1° next to the Brewster angle of the substrat(typically 56°) with standard EP³ (fig.1) or ArrayInspector (fig.2)

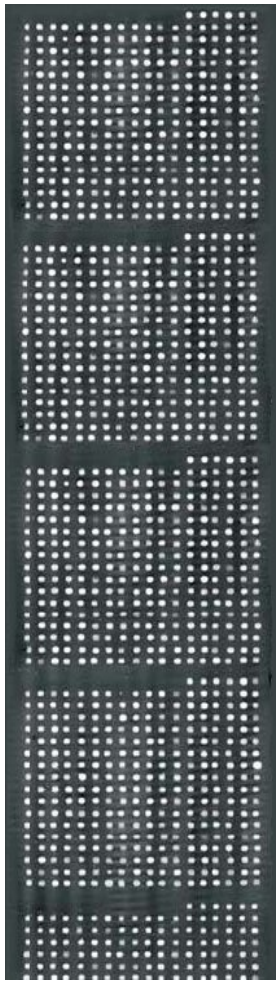

Fig. 2: Contrast image of the whole slide with se-veral DNA arrays (reso-lution < 10µm)

2. Angle of incidence (AOI) spectrum (fig.3) on activation layer (without reaction layer!)
3. Obtain effective refractive index n_s and effective extinction k_s of the effective substrate which is including the activation layer. Use n_s and k_s in the optical model (recipe) for further measurements.
4. Angle of incidence (AOI) spectrum on the reaction layer
5. Obtain parameters thickness d, refractive index n, and extinction k of the reaction layer. Use these parameters in the optical model (recipe) for calculation of maps.

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6. Record a delta-map and use the optical model to convert it into a thickness-map.
7. Mapping of single spots or small clusters of spots (fig.4)
8. Measurement of thickness in all ROIs on spots in the field of view simultane ously (fig.5)

Detailed investigation

Optical model and Data fit of micro arrays

The observables of the ellipsometer are the phase shift delta and the ratio of reflection coefficients psi. Delta and psi are simulated with the optical model of the micro array. The model contains free parameters, e.g. layer thickness d and refractive indices n and n_s of layer and substrate and extinction k of the layer. A mean square error (MSE) of measured and simulated delta and psi is made minimal by the program under variation of the free parameters. The parameters at minimal MSE are the fit results. Typically three different parameters can be obtained from a spectrum. Since here five parameters are desired, two different spectra are recorded: First the angle of incidence (AOI) spectrum (fig.3) on the activation layer around the spots is recorded.

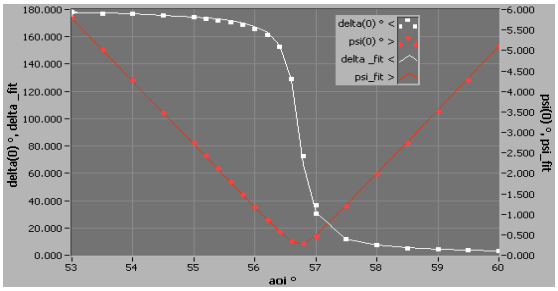

Fig. 3: ***Screenshot from EP³View 2.0 Software:*** Delta/Psi as angle of incidence (AOI) spectrum, 2-zone, recorded on activation layer, fit with optical model gives **refractive index n_s = 1.524 and k_s = 0.009**. Another spectrum recorded on one DNA-spot gives for the reaction layer: **thickness d = 5 nm, refractive index n = 1.64 and k = 0.07**.
In this case k is unequal zero due to the absorption of the fluorescence labeling of DNA. Imaging ellipsometry works on a label free reaction layer as well.

One obtains effective refractive index n_s and effective extinction k_s of the effective substrate which is including the activation layer. Then one records another spectrum on one of the spots. This second spectrum is fitted with n_s and k_s obtained from the first spectrum. Thickness d, refractive index n, and extinction k of the reaction layer are the results of this fitting. Use n and k of the reaction layer in the optical model. With this optical model finally a delta(-map) is converted into a thickness(-map) (fig.4a).

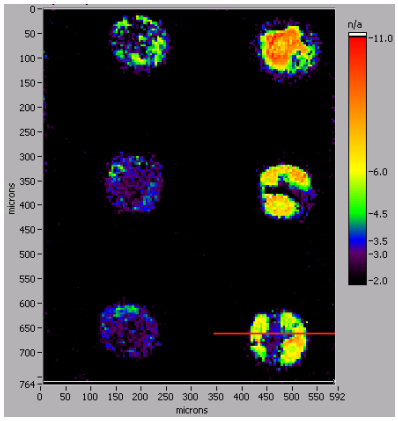

Fig. 4a: Six spots of DNA in a map (thickness in nm)

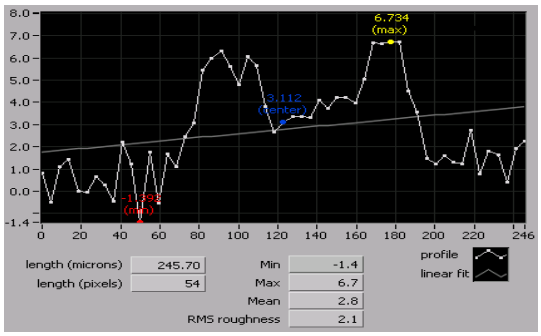

Fig. 4b: Cut along red line (fig.5a) through lower right spot (thickness in nm). Statistic information includes Mean, RMS roughness, Minimum, Maximum.

Results

1. Optical properties i.e. thickness (mass density), refractive index, extinction have been measured for the reaction layer in order to enable a thickness map (fig. 4a) of the micro array

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2. Irregularities of shape an thickness of the spots in a micro array have been observed with high lateral resolution in the thickness map (fig. 4a) and in the contrast image (fig. 1)

3. The variation of spot shape and mass density of all spots on the slide are obtained in a contrast image (fig.2) scanned by the Nanofilm's ArrayInspector

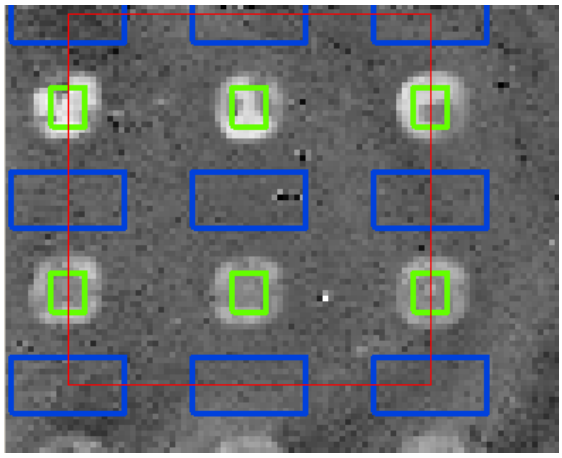

Fig.5: Grid of ROIs on the DNA array. One measurement gives a mean thickness for each ROI in the field of view.

Conclusion

Nanofilm's imaging ellipsometer EP3 offers both in one instrument: an accurate measurement of the optical properties of the reaction layer and a quick and high-resolution inspection of variation of shape

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