Characterisation of Thin Oligonucleotide Films on Epoxy-Coated Si using Imaging Ellipsometry.

In transparent regions when layers are thin, the ellipsometry parameter y is given to first order by

$$y = k\sum_{i=1}\eta_i \tag{1}$$

$$\eta_i = \frac{(\varepsilon_i - \varepsilon_1)(\varepsilon_i - \varepsilon_2)}{\varepsilon_i} t_i \tag{2}$$

Here ε_1 and ε_2 are the dielectric constants of the substrate and incident media and ε_i and t_i are the dielectric constant and thickness of the *i*th layer. The constant of proportionality k is dependent on ε_1 and ε_2, the angle of incidence θ, and the wavelength of the incident light λ.

Equation (1) and (2) can be rewritten

$$y = y_0 + ct \tag{3}$$

Here t is the thickness of a layer in the layer stack, c is constant that depends on $\varepsilon_1, \varepsilon_2, \theta, \lambda$ and ε of that layer, and y_0 is the effect of all other layers in the system.

Imaging ellipsometery was used to measure the ellipsometery parameter y for thin oligonucleotide films spotted onto epoxy-coated n-type silicon substrate. Measurements were made at $\theta = 70\,\text{deg}$ and $\lambda = 633\,\text{nm}$, and at three oligonucleotide concentrations.

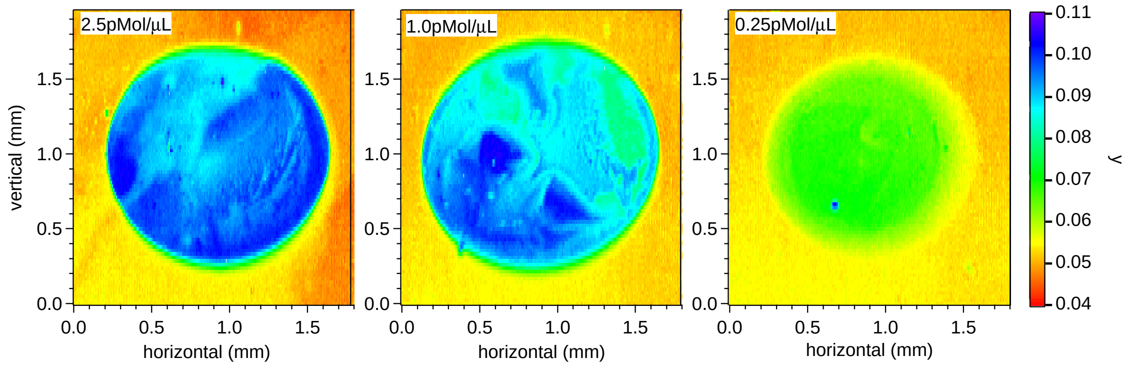

Figure 1. y images of three oligonucleotide spots.

Figure 1 show y images of three oligionucleotide spots at concentration of 2.5 $pMol/\mu L$, 1.0 $pMol/\mu L$ and 0.25 $pMol/\mu L$. Clearly the y value in spotted region is larger then that in the surrounding unspotted region.

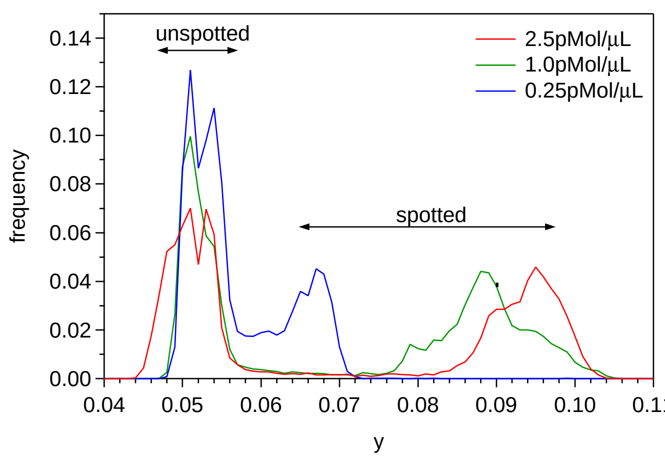

Figure 2. Distribution of y values over the images

Figure 2 show histograms giving the distribution of y values over the images shown in Figure 1. The distribution in all three cases is bimodal. The higher peak in the bimodal distribution corresponds to the spotted region in the image; the lower peak corresponds to the unspotted region. The modal y value in the spotted region is clearly correlated with the oligonucleotide concentration.

We assume that the *y* value in the spotted region can be described by

$$y_{spotted} = y_{unspotted} + ct\,, \tag{4}$$

or

$$\Delta y = ct\,, \tag{5}$$

where $y_{unspotted}$ is the average *y* value measured in the unspotted region. The constant *c* depends on dielectric constant of the overlayer which is unknown. Many organic materials have a refractive index of n=1.5. If we assume this as the refractive index of the oligonucleotide overlayer, then the constant *c* can be calculated numerically from the slope of *y* with thickness.

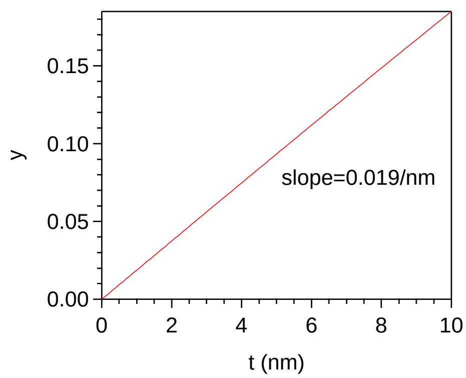

Figure 3. *y* as a function of layer thickness for $\varepsilon_1 = 1, \varepsilon_2 = 15$ $\varepsilon = (1.5)^2 = 2.25, \theta = 70$ deg and $\lambda = 633$ nm.

The thickness over the spotted region can be determined by subtracting the average value in the unspotted region from the *y* image in Figure 1, to give Δy, and dividing the resulting image by the slope *c* determined numerically.

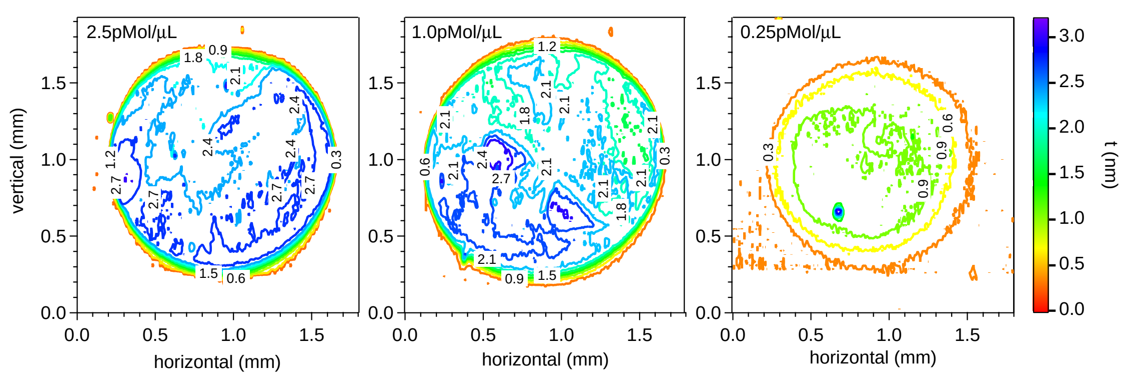

Figure 4. Thicknesses (in nm) of the oligonucleotide thin films

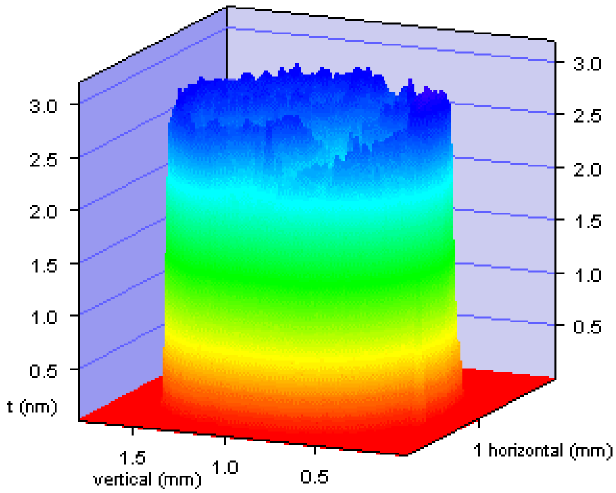

Figure 5 3D view of 2.5 *pMol* / *μL* oligionucleotide spot showing thickness variation.