

For incident light polarized perpendicular to the plane of incidence, the detected intensity variation at a given pixel in the image relates to the average height of the fluorophore and the angle of incidence as follows:

$$I = A(|1 + r^{TE} e^{i\phi(H)}|)^2 + B \quad (1)$$

where

A - accounts for variation in detected intensity due to factors including mean excitation laser intensity, fluorophore density, efficiency of emitted photon detection, etc..

B - is an offset parameter that accounts for background fluorescence in the sample images.

H - is the position above the silicon oxide layer

$\phi(H)$ - the phase difference of the direct and reflected light at axial position H given by:

$$\phi(H) = \frac{4\pi}{\lambda} (n_b H \cos \theta_b) \quad (2)$$

r^{TE} - the effective Fresnel coefficient obtained from the transfer matrix m^{TE} according to:

$$r^{TE} = \frac{(m_{11}^{TE} + m_{12}^{TE} p_0) p_2 + (m_{21}^{TE} - m_{22}^{TE} p_0)}{(m_{11}^{TE} + m_{12}^{TE} p_0) p_2 + (m_{21}^{TE} + m_{22}^{TE} p_0)} \quad (3)$$

where

$$m_{11}^{TE} = \cos(k_{0x} d_{ox} \cos \theta_{ox}) \quad (4)$$

$$m_{12}^{TE} = \frac{-i}{p_1} \sin(k_{0x} d_{ox} \cos \theta_{ox}) \quad (5)$$

$$m_{21}^{TE} = -i p_1 \sin(k_{0x} d_{ox} \cos \theta_{ox}) \quad (6)$$

$$m_{22}^{TE} = \cos(k_{0x} d_{ox} \cos \theta_{ox}) \quad (7)$$

$$p_0 = n_{Si} \cos \theta_{Si}, p_1 = n_{ox} \cos \theta_{ox}, p_2 = n_b \cos \theta_b \quad (8)$$

$$k_i = \frac{2\pi n_i}{\lambda} \quad (9)$$

k_i - wavenumber in the given material

n_{Si} - refractive index of silicon

n_{ox} - refractive index of the oxide layer

n_b - refractive index of the sample

θ_{Si} - angle of incidence in the silicon

θ_{ox} - angle of incidence in the oxide layer

θ_b - angle of incidence in the sample

By rewriting the effective Fresnel coefficient as a complex number:

$$r^{TE} = c + id \quad (10)$$

Equation 1 can then be rewritten as:

$$I = A(1 + 2c \cos \phi(H) - 2d \sin \phi(H) + c^2 + d^2) + B \quad (11)$$

Not only can equation 11 be computed about 10 times faster than equation 1, it can also be used to derive partial derivatives needed for Levenberg-Marquard non-linear least square curve fitting, using

$$f = \frac{d}{dH} \phi(H) = \frac{4\pi n_b \cos \theta_b}{\lambda} \quad (12)$$

Partial derivative of equation 11 for H:

$$\frac{d}{dH}(I) = -2Af(c \sin \phi(H) + d \cos \phi(H)) \quad (13)$$