An implementation of deconvolution software for wide field microscopy

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Abstract

Presented here are results of a Bachelor thesis project implementing deconvolution software for widefield microscopy at the Quantitative Imaging group at Delft University of Technology. The lens model presented by S.F. Gibson et al is used to calculate optical aberrations [1]. We evaluate the effectiveness of the software by simulating a microscopic image of a test object and compare the restored object to original test object. We conclude by observing the effectiveness of the implemented restoration software.

Deconvolution

Assume a simple linear relationship between an object x and its image y:

$$y = x \otimes P + n \tag{1}$$

Here, $\otimes$ represents a convolution operation, n is an noise term and P stands for the point spead function of the imaging system. To find a restoration filter H which gives us the best estimate of the object $\hat{X} = HY$, we define

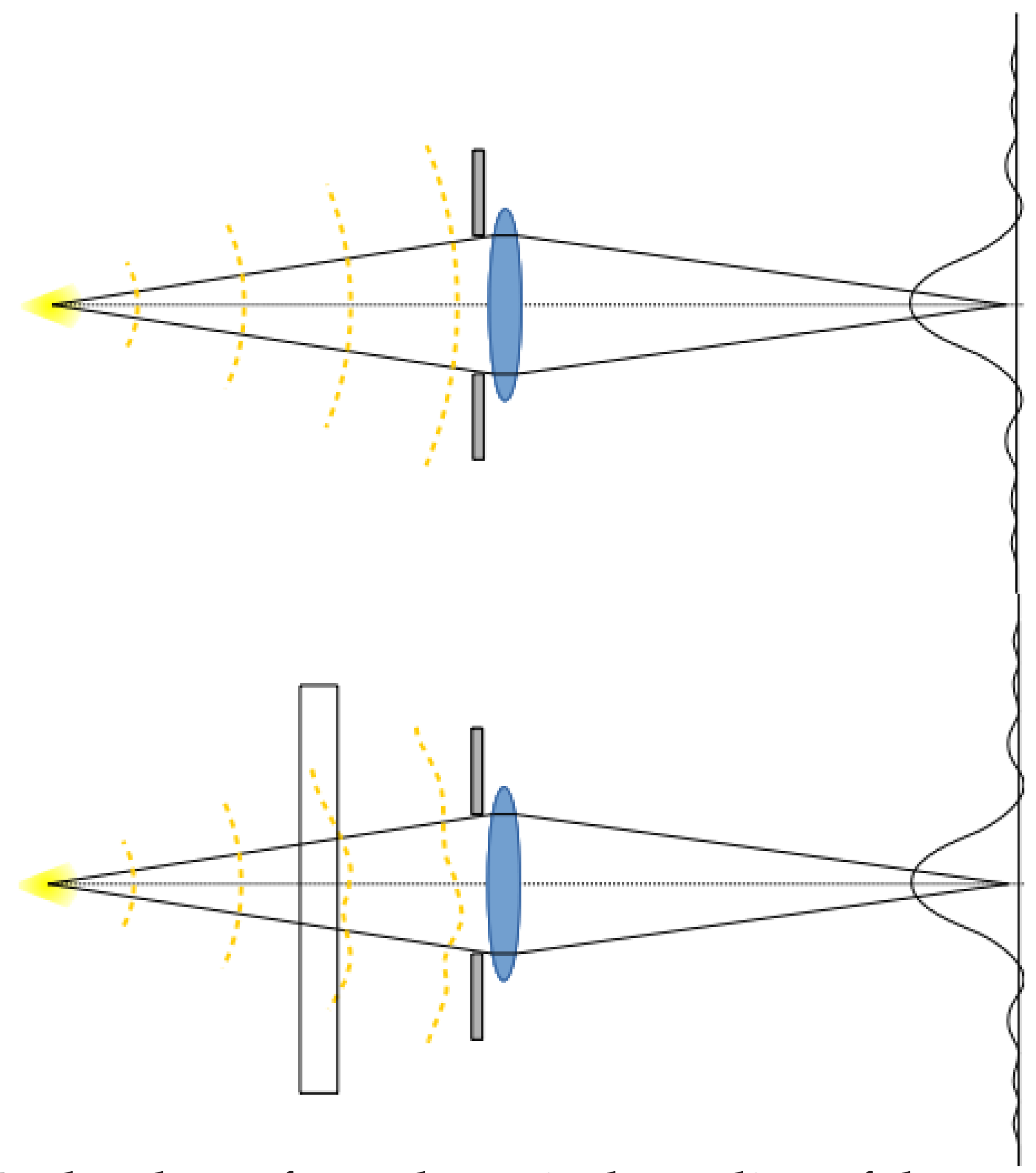
$$\chi = \mid O\hat{X} - Y \mid^2 + \gamma \mid \hat{X} \mid^2 \tag{2}$$

Here, $O = \mathcal{F}(P)$ is the optical transfer function, $X = \mathcal{F}(x)$ and $Y = \mathcal{F}(y)$, where $\mathcal{F}$ is the Fourier transform operator. The best estimate $\hat{X}$ minimizes χ. The term γ is a regularization term and keeps the minimum away from noisy solutions. We find

$$\hat{X} = (OO^T + \gamma I)^{-1} O^T Y \tag{3}$$

How a suitable value for γ is found is described in the regularization section. We are left with finding the point spread function of our microsope. This can be measured but it was desided in this study to calculate it theoretically.

Point Spread Function



Under design conditions the image I of a point source imaged by a single apature and lens is given by

$$I(X,Y,Z) = \mid \frac{C}{Z} \int_0^1 J_0(ka\rho \frac{\sqrt{X^2+Y^2}}{Z}) \rho d\rho \mid^2 \tag{4}$$

Where ρ is the normalized radius r/R in the aparture of radius R. Further, $k = 2\pi/\lambda$, where λ is the wavelength of the light used. J_0 is the zeroth order Bessel function

When the phase and intenstiy of the light in the aparture is not constant (that is, in the presence of aberrations), a more generalized function is given by

$$I(X,Y,Z) = \mid \frac{C}{Z} \int_0^1 J_0(ka\rho \frac{\sqrt{X^2+Y^2}}{Z}) exp(iW(\rho)) \rho d\rho \mid^2 \tag{5}$$

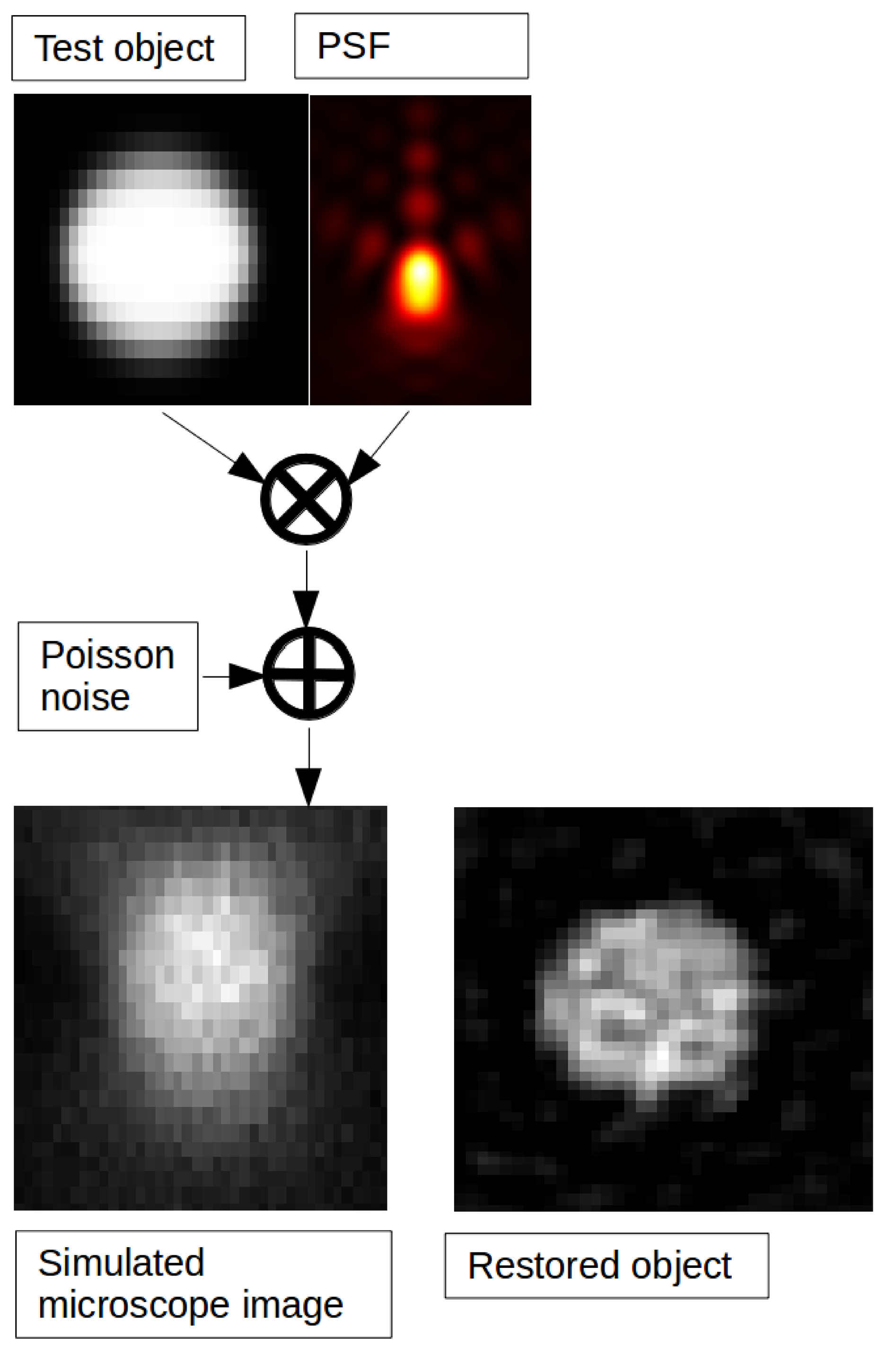
Here the function $W(\rho)$ gives the difference in phase between actual and nominal, aberration free, conditions

In the above formulas, a is the radius of the projection of the aperture on the back focal plane of the objective lens. Under the sine condition, it can be shown that

$$a = \frac{Z^* NA}{\sqrt{M^2 - NA^2}} \tag{6}$$

Here Z^* is the distance between the projection plane and the aperture under design conditions, NA is the numeric aperture and M is the magnification of the objective lens.

Software evaluation



The PSF is calculated by numerical integration of equation 5. A simulated microscope image is effectively restored.

Regularization

We employ a cross validation method to find γ by finding a minimum value in the function

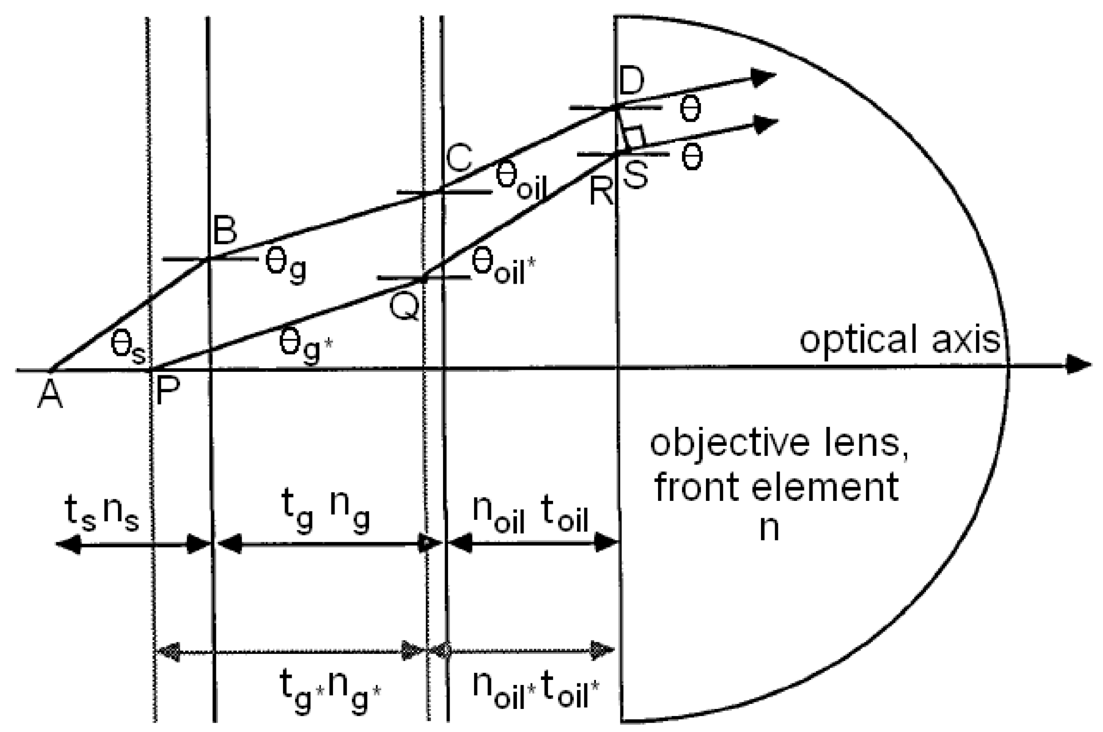
$$CV(\gamma) = \frac{\sum_\omega \frac{\gamma^2 |Y(\omega)|^2}{(|O(\omega)|^2+\gamma)^2}}{\left(\sum_\omega \frac{\gamma}{|O(\omega)|^2+\gamma}\right)^2} \tag{8}$$

A minimum value is found with Brents minimization algorithm.

References

1. *Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy*

Aberration



In this study, the lens model presented by S.F. Gibson et al ([1]) was used to calculate aberrations in the wavefront. We consider the path length between the object and detector under nominal conditions and the path length under aberrating conditions, e.g. when the cover slip thickness or the refractive index of the immersion oil is different then specified. The approximate path length difference between these conditions is given by

$$OPD \approx n_s t_s \left[1 - \left(\frac{NA\rho}{n_s}\right)^2\right]^{1/2} + n_g t_g \left[1 - \left(\frac{NA\rho}{n_g}\right)^2\right]^{1/2} + n_{oil} t_{oil} \left[1 - \left(\frac{NA\rho}{n_{oil}}\right)^2\right]^{1/2} - n_{g^*} t_{g^*} \left[1 - \left(\frac{NA\rho}{n_{g^*}}\right)^2\right]^{1/2} - n_{oil^*} t_{oil^*} \left[1 - \left(\frac{NA\rho}{n_{oil^*}}\right)^2\right]^{1/2} \tag{7}$$

t_s, t_g and t_{oil} are the thickness of respectively the specimen layer, cover slip layer and the immersion oil layer. n_s, n_g and n_{oil} are the refraction indices of these layers. The symbols with a * super script denote the same things when the system is working under design conditions. The function $W(\rho)$ is now given by $W(\rho) = OPD * k = \frac{2\pi OPD}{\lambda}$