Depth of focus for digital microscopy

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The optical transfer function (OTF) offer a sensible approach to the depth of focus for digital microscopy. Defocusing results in breakdown of OTF in the middle of the transfer spectrum. The resulting formula for depth of focus is independent from the sensor pixel size.

1 Introduction

The human eye can accommodate to defocused details of image and classical formulas for depth of focus take into account this ability. A digital sensor can't do so. Therefore the classical formulas like Bereks formula [1] are not valid for digital microscopy.

2 New approach to the depth of focus

The value of OTF describes the image quality at incoherent conditions. When the optical system is defocused, this value decreases. At the value of zero the depth of focus is reached. Due to Duffieuxs integral [2] OTF is

$$OTF(2R_x) = \frac{1}{\pi} \iint\limits_{\substack{(x-R_x)^2+y^2\le 1\\(x+R_x)^2+y^2\le 1}} e^{\frac{2\pi i}{\lambda}\left(F(x-R_x)-F(x+R_x)\right)} dx dy$$

Here are x and y reduced coordinates of pupil, Rx is reduced spatial frequency and F wavefront aberration. The area of integration and its approximation are shown in figure 1. The space of the approximation is equal to the space of the area of integration.

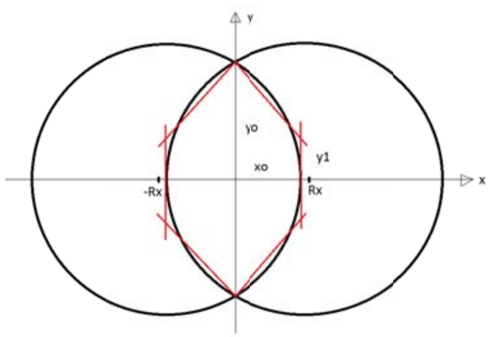

Figure 1: Area of integration and its approximation

Defocus of Δr results in wave aberration of

$$F(x) = -\frac{\Delta r}{2} \cdot NA^2 \cdot x^2$$

NA is the numerical aperture.

With respect to this formula and to the symmetry the equation for OTF can be simplfyed to

$$OTF(2R_x) = \frac{1}{\pi} \iint\limits_{\substack{(x-R_x)^2+y^2\le 1\\(x+R_x)^2+y^2\le 1}} \cos \alpha x \, dx dy$$

with

$$\alpha = \frac{4\pi}{\lambda} \Delta r \cdot NA^{2 \cdot R_x}$$

Further simplification of this integral are not possible because of the complicated area of integration. But this is possible when the area was approximated as described above (figure 1):

$$OTF(2R_x) = \frac{4}{\pi}\left[\frac{y_1}{\alpha}\sin \alpha x_0 - \frac{\gamma}{\alpha^2}(1-\cos \alpha x_0)\right]$$

Here is y1 the height of the rectangular part of approximation area, xo the half width of the area of integration and γ the slope of the edge of approximation area.

3 Calculation of the depth of focus

When Δr is depth of focus, OTF(2Rx) must be zero. Because of this a further simplification is possible:

$$\frac{\alpha \cdot y_1}{\gamma} = \tan \frac{\alpha}{2} x_0$$

This formula is a condition for Δr to be depth of focus. Because of

$$\frac{\alpha}{2} x_0 \approx \frac{\pi}{2}$$

it is sensible to approximate tangent in the formula to

$$\tan \frac{\alpha}{2} x_0 \approx \left[\frac{\pi}{2} - \frac{\alpha}{2} x_0\right]^{-1}$$

Due to this approximation a transformation of the formula into a quadratic equation is possible

$$0 = \alpha^2 - \frac{\pi}{x_0}\alpha + \frac{2\gamma}{y_1 \cdot x_0}$$

The solution of this equation is

$$\alpha = \frac{\pi}{2x_0}\left(1 + \sqrt{1 - \frac{8}{\pi^2}\left(1 - \frac{y_0}{y_1}\right)}\right)$$

where yo is the height of the area of integration. Because yo/y1 is almost the linear function t(Rx) another approximation is sensible (please see table 1).

$$t(R_x) = 1.754 + R_x \cdot 1.273$$

R_x	y_1	y_0	y_0/y_1	$y_0/y_1 - t(R_x)$
0.0	0.57	1.00	1.754	0.000
0.1	0.53	0.99	1.868	-0.013
0.2	0.49	0.98	2.000	-0.009
0.3	0.45	0.95	2.111	-0.025
0.4	0.40	0.92	2.300	0.037
0.5	0.36	0.87	2.417	0.027
0.6	0.32	0.80	2.500	-0.018
0.7	0.27	0.71	2.630	-0.015
0.8	0.22	0.60	2.727	-0.045
0.9	0.15	0.44	2.933	0.033

Table 1: yo/y1 as a linear function

Due to this approximation the solution of the quadratic equation can be transformed to

$$\Delta r = \frac{\lambda}{8NA^2} \cdot \frac{1 + \sqrt{1.611 + 1.032 \cdot R_x}}{R_x \cdot (1 - R_x)}$$

Obviously Δr as the depth of focus is a function of Rx. This means that each spatial frequency Rx has his own depth of focus. The “global” depth of focus can be only the minimal value of Δr over all spatial frequencies Rx. Therefore the condition

$$\frac{d}{dR_x} = 0$$

must be fulfilled. To do so it is necessary to satisfy the quadratic equation

$$0 = R_x^2 + 1.748 \cdot R_x - 1.041$$

The solution of this equation is Rx = 0.469 and the corresponding Δr is

$$\Delta r = 1.228 \frac{\lambda}{NA^2}$$

This is the resulting formula for depth of focus.

Figure 2 show the MTF (real part of OTF) for a sequence of defocus from the focal position to the depth of focus.

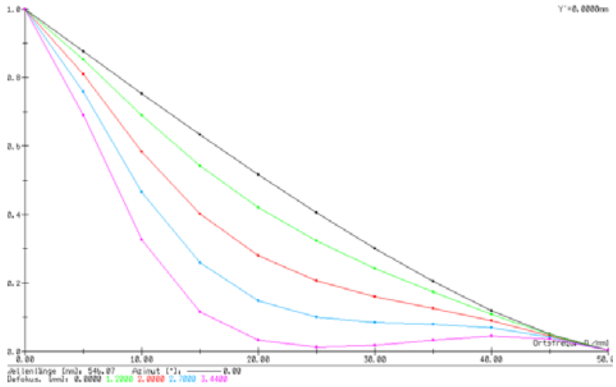

Figure 2: Sequence of defocus, NA=0.014, λ=546nm

In microscopy it is important to transfer this image related formula for depth of focus into the object space

$$\Delta\rho = 1.228 \cdot \frac{n}{\beta^2} \cdot \frac{\lambda}{NA^2} = 1.228 \cdot \frac{n \cdot \lambda}{na^2}$$

Here is Δρ the depth of focus in object space n the index of refraction of object space medium, β the magnification and na the numerical aperture on object side.

4 Independence from pixel size

With respect to the Nyquist frequency the pixel size of image sensor should be small enough to resolve limit spatial frequency of optical system. Of course such a sensor would resolve the middle of spatial frequency spectrum too. Because of this and the circumstance that in the case of defocus MTF breaks down at the middle of the spectrum, the formula for depth of focus is independent from pixel size.

Usually the values of the new formula are much smaller than the values of Bereks formula [1]. Only for high magnification and high numerical aperture these values are almost comparable. Therefore depth of focus for digital microscopy is usually smaller then for conventional microscopy

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Literatur

[1] ISO 19012-1, Beuth Verlag, 2010

[2] *H.Haferkorn, „Bewertung optischer Systeme“, VEB Deutscher Verlag der Wissenschaften, Berlin 1986*