Estimating the diffraction-limited resolution of your objective lens.

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The following focal volume approximations are based on Gaussian functions fit to integral representation of the electric field near the focus of a diffraction-limited focus obtained from the formalism of Richard and Wolf (1959, Proc. Royal Soc. A, 358 - 379).

Note that to obtain a "diffraction-limited" focus the beam of light entering the back of the lens must be of ~equal intensity across the lens aperture. Since laser beams are usually Gaussian in X and Y (brightest at the center of decreasing in intensity at the periphery) this condition is usually accomplished by expanding the beam so that only the center is used. A practical rule is that if the 1/e diameter of the laser beam is about the diameter of the back aperture, the spot is for all practical purposes, diffraction-limited (and the equations below are good approximations to the size of the focused spot).

ω is the 1/e radius

For the $1/e^2$ radius multiply by $\sqrt{2}$

For FWHM by $2\sqrt{\ln 2}$ = 1.665.

m = order of excitation – i.e. m = 1 for conventional excitation, m = 2 for 2 photon excitation

n_i = index of refraction of the immersion medium

LATERAL RADIUS (average of widths along and perpendicular to polarization axes)

For low NA (0.1 to ~0.7):
(Max error = 7% at 1.4 NA)

$$\omega_{xy} = \frac{0.32\lambda}{\sqrt{m}\,NA}$$

High NA (0.7 to 1.4)
(~1% error max.)

$$\omega_{xy} = \frac{0.325\lambda}{\sqrt{m}\,NA^{0.91}}$$

AXIAL RADIUS

note: α = arcsin(NA/n) in radians

$$\omega_z = \frac{0.266\lambda}{n_i\sqrt{m}\sin^2(\alpha/2)} = \frac{0.532\lambda}{\sqrt{m}}\left(\frac{1}{n_i - \sqrt{n_i^2 - NA^2}}\right)$$

OTHER USEFUL STUFF:

Area = $\pi\,\omega_{xy}^2$

Volume = $\pi^{3/2}\,\omega_{xy}^2\,\omega_z$

$$Area_{1p} = \frac{0.094\lambda^2}{NA^2}$$ integrated area at the focus (z = 0) as a function of wavelength and NA

$$EPD = 2NAf_{obs} = 2NA\frac{f_{tubelens}}{Mag}$$ (*EPD* = Entrance pupil diameter = "back aperture")

$$5\times10^{12}\lambda\ photons/mW \qquad (\lambda\ in\ nm)$$ Number of photons per mW