DRBIO
An NIH Research Resource

Application Note #1

Estimating the focused spot size of your objective lens in a laser scanning microscope.

(W.R. Zipfel. Cornell University)

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).

If the lens back aperture is underfilled (in a practical sense – if the beam to your eye looks much smaller then the opening of the objective lenses) the relationships below do not hold and the spot can be much larger -- i.e. you're not operating at the diffraction limit and not achieving the resolution possible for the numerical aperture (NA) of the objective. This situation is covered on page 2.

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

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

$$\omega_{xy} = \frac{0.31\lambda}{\sqrt{m}\,NA}$$

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

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

AXIAL RADIUS

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

α = arcsin(NA/n) in radians. ω is the 1/e radius. For the 1/e^2 radius multiply by $\sqrt{2}$. For the FWHM multiply by $2\sqrt{\ln 2}$ = 1.665.
m = order of excitation: m = 1 for conventional excitation, m = 2 for 2 photon excitation, etc.