Estimating the diffraction-limited resolution of your objective lens.
(W. Zipfel, Biomedical Engineering/Applied Physics, 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).

\( \omega \) 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):  
\[
\omega_x = \frac{0.32\lambda}{\sqrt{m NA}}
\]

High NA (0.7 to 1.4):  
\[
\omega_x = \frac{0.325\lambda}{\sqrt{m NA^{0.91}}}
\]

AXIAL RADIUS

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

OTHER USEFUL STUFF:

Area = \( \pi \omega_{xy}^2 \)

Volume = \( \pi^{3/2} \omega_{xy}^2 \omega_z \)

\( Area_{int} = \frac{0.094\lambda^2}{NA^2} \) 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”)

\( 5 \times 10^{12} \lambda \) photons / mW \((\lambda \text{ in nm})\)  
Number of photons per mW