<u>Formulas used:</u>

Axial (res_z^o) theoretical resolution value used for single point scanning multiphoton excitation microscopes is calculated as defined in Zipfel, W.R., Willams, R.M. & Webb, W.W. Nonlinear magic: multiphoton microscopy in the biosciences, Nature Biotechnology 21, 1369–1377 (2003):

$$res_z^o = \frac{0.626 * \lambda_{ex}}{n - \sqrt{n^2 - NA^2}}$$

NA: numerical aperture, λ_{ex}: excitation wavelength, n: refractive index of the lens immersion & mounting media

Z-axis profile is fitted using ImageJ Gaussian Curve Fitter and the following formula $y = a + (b - a) * e^{\frac{-(x-c)^2}{2d^2}}$ (Gaussian fitting).

Measured axial resolution (Full Width at Half Maximum, FWHM) value is derived using $\text{FWHM} = 2d\sqrt{2ln(2)}$

Compliance with the Shannon-Nyquist criterion for k photon excitation uses the following formula for Shannon-Nyquist distances calculation (and a fixed k value of 2):

$$\alpha = \arcsin(\frac{NA}{n})$$

$$\Delta_z = \frac{\lambda_{ex}}{4.n.\ (1 - cos\ (\alpha))}$$